University of Alberta

Effects of Surfactants on Microbial Adhesion to Solid and Semi-Solid Surfaces

by

Patricia Louise Stelmack ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of Master of Science

Department of Chemical and Materials Engineering

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Patricia Stelmack
10045 - 83 Avenue #212
Edmonton, Alberta
T6E 2C3

April 18, 1997

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Effects of Surfactants on Microbial Adhesion to Solid and Semi-Solid Surfaces submitted by Patricia Louise Stelmack in partial fulfillment of the requirements for the degree of Master of Science.

MAPickard

Dr. M.A. Pickard, supervisor

Dr. M.R. Gray, co-supervisor

WMcCaffrey

Dr. W. McCaffrey

Dr. J. Foght

April 18, 1997

ABSTRACT

The use of surfactants has been proposed as a means to increase the bioavailability of virtually insoluble contaminants. Many research groups, however, have studied the effects of surfactants on the biodegradation of hydrocarbons, and the conclusions stemming from these studies have ranged from inhibited biodegradation in the presence of surfactants to enhanced biodegradation in the presence of surfactants. Thus, there is a need to determine the mechanism involved with surfactant-microorganism-hydrocarbon interactions.

The goal of this study was to determine the effects of the presence of surfactants on the adhesion of a *Rhodococcus* species and a *Pseudomonas* species to solid and semi-solid surfaces. Experiments were designed and carried out to determine the effects of low concentrations of surfactants on initial adhesion of the microorganisms to hydrocarbons, adhesion of the microorganisms already attached to hydrocarbons, growth of the microorganisms on solid anthracene, and migration of the microorganisms through a packed column.

It was found that both species were able to adhere to semi-solid tars, anthracene, and glass. Further, the presence of either a nonionic or an anionic surfactant at a concentration of half its CMC will inhibit adhesion to semi-solid tars, promote the removal of microorganisms from semi-solid tars, and inhibit adhesion to solid anthracene. Finally, the presence of surfactant will inhibit the adhesion of the *Rhodococcus* species to glass beads in a packed column, however the *Pseudomonas* species would not adhere to the glass beads, regardless of the presence or absence of surfactants.

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1.0 INTRODUCTION

Biodegradation has been identified as an environmentally sound and economically viable method for reclaiming contaminated sites. One of the major problems with this method, however, is that microorganisms prefer to biodegrade target contaminants in the aqueous phase, while many of these contaminants are virtually insoluble in water. Thus, in order to make the biodegradation process more efficient, methods that enhance the bioavailability of such contaminants must be developed.

The use of surfactants has been proposed as a means to render hydrophobic contaminants more soluble in water. Many studies have been carried out in order to determine the effects of the presence of various surfactants, mostly nonionic and some anionic, on the biodegradation of different organic contaminants. The conclusions reached in these studies have varied markedly. Some researchers have claimed that the presence of surfactants enhances biodegradation efforts, while others have claimed that the presence of surfactants inhibits biodegradation efforts. These conflicting views reflect the fact that there is a need to understand the mechanism involved when surfactants are added to a contaminated soil system.

The first step in understanding this mechanism is to identify the steps taken by the microorganisms in order to degrade hydrophobic contaminants in the nonaqueous phase. The rate limiting step in this process seems to be the adhesion of the microorganisms to the organic substrate. This appears to be regulated not only by the ability of the microorganisms to contact the interface between the aqueous and nonaqueous phases, but also by the nature of their cell walls.

This investigation has focused on determining the effects of the presence of surfactants on the adhesion of microorganisms to surfaces. Both a nonionic surfactant and an anionic surfactant were chosen in order to determine whether surfactant charge has any effect on microbial adhesion. Similarly, both a Gram positive microorganism and a Gram negative microorganism were selected in order to determine whether the adhesion capabilities of different strains are related to cell wall structures. Experiments were set up to determine the effects of the presence of surfactants on the initial adhesion of the microorganisms to organic substrates, the effects of the addition of surfactants to a system in which the microorganisms were previously allowed to adhere to organic substrates, the effects of the presence of surfactants on the adhesion of the microorganisms to a solid carbon source for the purpose of colonization, and the effects of the presence of surfactants on the adhesion of microorganisms within a packed column. Controls in which no surfactant was present were set up for comparison purposes.

2.0 LITERATURE SURVEY

2.1 BIODEGRADATION OF HYDROCARBONS

Industrial chemicals such as hydrocarbons have been released into the environment as a result of mechanical failure, incineration practices, corrosion, leakage, accidental spillage, or improper disposal practices (Vanloocke *et al.* 1975; Sitar *et al.* 1987; Cerniglia 1992). Many of these chemicals remain in the ground and continue to accumulate as more and more releases occur. Government standards that regulate the allowable chemical concentrations in soil have become more rigid in recent years, and site remediation has become necessary. Biodegradation is one method that is under investigation as a possible means of site reclamation.

2.1.1 Migration of Hydrocarbons through Soil

Hydrocarbon liquids are hydrophobic substances, and when they are released into soil at high concentrations, they are transported as a separate liquid phase from water. Nonaqueous phase liquids that are released into the soil sink towards the water table, as shown in Figure 2.1 (Sitar *et al.* 1987). Nonaqueous phase liquids that are heavier than water continue to migrate downward through the water table, and collect on top of a low permeability layer below the water table. Conversely, nonaqueous phase liquids that are lighter than water start to spread laterally when they reach the water table.

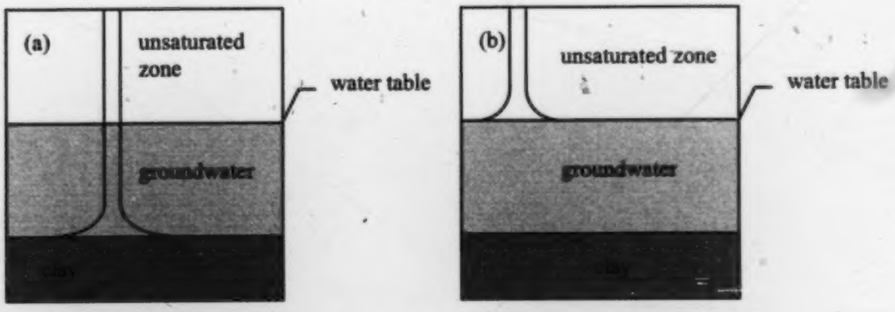


Figure 2.1: Distribution of Nonaqueous Phase Liquids in Soil; (a) Liquids Heavier than Water; (b) Liquids Lighter than Water.

Because of their hydrophobic nature, nonaqueous phase liquids tend to sorb to soil as they spread through the soil matrix. Eventually, the concentration of hydrocarbons in the soil phase reaches an equilibrium with the surrounding liquids (Dzombak and Luthy 1984). This equilibrium takes a long time to attain, which may explain why nonaqueous phase liquids become more and more difficult to remove from soil particles as time passes (Shuttleworth and Cerniglia 1995). In fact, it has been found that the extent of biodegradation is inversely proportional to contaminant age (Weissenfels *et al.* 1992; Ghosh *et al.* 1994). It should also be noted that in general the aqueous solubility of hydrocarbons is inversely proportional to their molecular weight (Gauger *et al.* 1990; Park *et al.* 1990).

2.1.2 Ecological Effects of Hydrocarbons

Once deposited, hydrocarbons can remain in soil for long periods of time. As more and more deposits are made, hydrocarbons continue to accumulate. These deposits may then limit the transport of water and other nutrients through the soil, which limits plant growth and animal activity. This, in turn, leads to a decrease in microbial activity (Morgan and Watkinson 1989).

Humans may exhibit health defects as a result of exposure to certain hydrocarbons and halogenated compounds. These deleterious health effects include skin irritations, seizures, breathing abnormalities, liver damage, kidney failure, and cancer. As a result, many hydrophobic organic materials have been placed on the United States Environmental Protection Agency (USEPA) First Priority List of 100 Hazardous Substances (Sittig 1991). Some of these substances are listed in Table 2.1.

Table 2.1: Selected Organic Materials on the USEPA First Priority List of 100 Hazardous Substances

Benzene	Benzo(a)anthracene	Benzo(a)pyrene
Benzo(b)fluoranthene	Chrysene	Dibenzo(a,h)anthracene
Ethylbenzene	Fluoranthene	Indeno(1,2,3-cd)pyrene
Naphthalene	Phenanthrene	Phenol
Polychlorinated biphenyls	Toluene	Xylenes

2.1.3 Advantages of Biodegradation

One key advantage of biodegradation over conventional remediation treatments, such as incineration or pumping and treating, is its economic viability (Heitzer and Sayler 1993). Biodegradation takes place on site, which eliminates the cost of transporting contaminated soils. Other costs associated with this method, such as processing, maintenance, and staffing, are competitive with other more traditional remediation methods (Loehr 1992).

In many cases, biodegradation efforts are just as efficient at removing contaminants as traditional remediation methods, without the addition of new contaminants to the soil. This is because biodegradation is an extension of a natural process which can be accelerated by optimizing key variables (Loehr 1992). Complete biodegradation leads to the formation of CO_2 and biomass.

Another advantage of biodegradation is its environmental soundness. The lack of transportation requirements means that vehicle emissions are kept to a minimum. Traditional remediation methods also tend to create new waste, such as incineration residues, which is not the case with biodegradation (Heitzer and Sayler 1993).

2.1.4 Disadvantages of Biodegradation

Biodegradation effectiveness is directly related to the behavior of the microorganisms that break down hydrocarbons. Many microorganisms will only degrade contaminants under certain conditions (Shuttleworth and Cerniglia 1995). Thus, biodegradation may not be possible if the contaminants are toxic to the microorganisms, the soil is either too acidic or too alkaline, there is a shortage of essential nutrients such as nitrogen, phosphorus or potassium, or there is a lack of oxygen or other electron acceptor (Loehr 1992).

Another limitation of biodegradation is that many hydrocarbons are not very accessible to microorganisms. This is due to the fact that biodegradation takes place in the aqueous phase (Wodzinski and Bertolini 1972; Wodzinski and Coyle 1974), while heavy hydrocarbons are virtually insoluble in water. It has also been suggested that the presence of microorganisms may actually decrease the concentration of dissolved hydrocarbons

(Thomas *et al.* 1986). One explanation for this observation is that the ability of hydrocarbons to dissolve from the nonaqueous phase into the aqueous phase as more and more hydrocarbons are metabolized can become mass transfer limited. Once the rate of degradation exceeds the rate of dissolution, then the aqueous phase concentration will fall well below saturation levels.

In order to degrade hydrocarbons effectively, the microorganisms must be able to come into contact with the hydrocarbons. Thus, microorganisms must be able to move through the soil matrix in order to reach these compounds. As they move, however, the microbial culture may lose viability because of starvation, predation, lysis, or parasitism (Gannon *et al.* 1991). The use of bioreactors has been proposed as a means to overcome this problem. The advantages of this method are that the contaminated soil is slurried with water at an optimum temperature, the slurry retention time may be varied, a suitable microbial culture may be used, and the water may be recycled after the treated material passes through a water separation system (Morgan and Watkinson 1989).

2.1.5 Pathways of Biodegradation

The major categories of hydrocarbons in contaminated soil are *n*-alkanes, branched alkanes, cycloalkanes, and aromatic hydrocarbons. The *n*-alkanes are considered to be the most readily degraded of the hydrocarbons. The primary pathway of biodegradation of *n*-alkanes, shown in Figure 2.2, proceeds by oxidation at the terminal methyl group to form a primary alcohol (Britton 1984). The primary alcohol is oxidized to an aldehyde, which is then oxidized to a carboxylic acid (Atlas 1981). The *n*-alkane is oxidized in the lipid bilayer of the microorganism to form the primary alcohol, and then the primary alcohol diffuses laterally in the membrane until it is oxidized to form the aldehyde. The aldehyde is then oxidized in either the membrane or the cytoplasm to form the carboxylic acid (Britton 1984). Finally, oxidation of the carboxylic acid occurs in the cytoplasm as units of acetyl coenzyme A are formed, with the eventual liberation of CO_2.

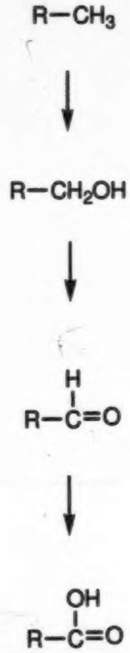
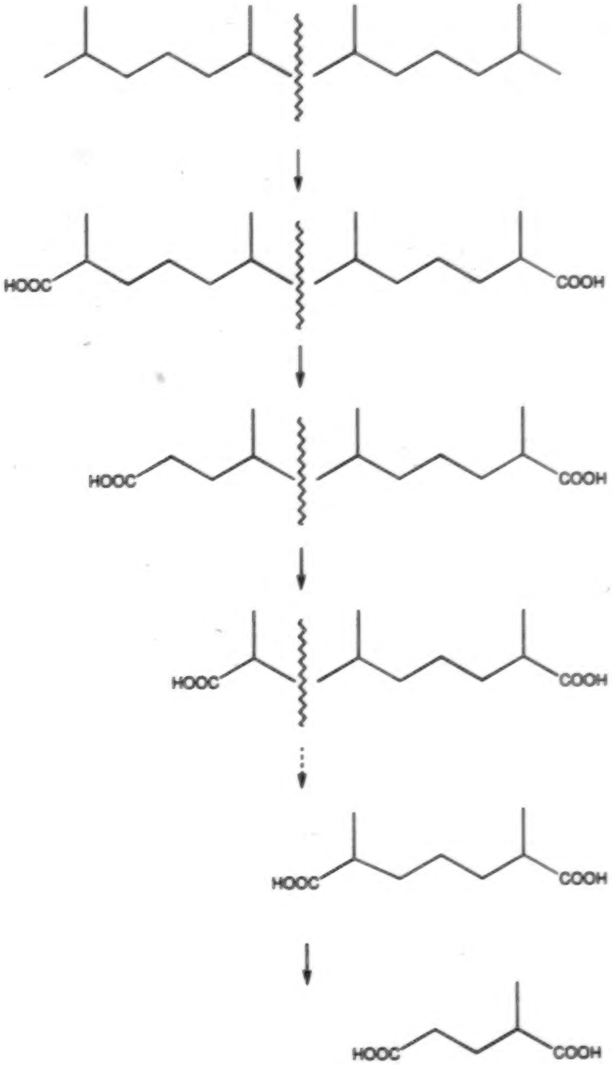


Figure 2.2: Biodegradation Pathway of *n*-Alkanes

Branched alkanes are more resistant to biodegradation than *n*-alkanes. Biodegradation occurs primarily by diterminal oxidation, as a dicarboxylic acid is formed (Atlas 1981). The dicarboxylic acid is then further oxidized by carbon cleavages. Figure 2.3 shows the biodegradation pathway of a branched alkane in which oxidation occurs via alternate cleavages of propionyl coenzyme A and acetyl coenzyme A units. There are few reports on biodegradation of more complex branched alkanes because these compounds are extremely resistant to biodegradation (Britton 1984).


HOOC
COOH
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COOH
HOOC
COOH
HOOC
COOH
HOOC
COOH

Figure 2.3: Biodegradation Pathway of Branched Alkanes

Cycloalkanes are also quite resistant to biodegradation. The first step in their biodegradation pathway is oxidation by either direct oxidation or co-oxidation to form a primary alcohol. Once this has occurred, biodegradation proceeds with ring cleavage (Atlas 1981). Biodegradation of substituted cycloalkanes seems to occur more readily than the biodegradation of the unsubstituted forms, especially if an *n*-alkane substituent of adequate chain length is present (Trudgill 1984). In such cases, biodegradation occurs on the substituted portion first, leading to the formation of cyclohexane carboxylic acid (Atlas 1981). The biodegradation pathway of cyclohexane carboxylic acid is shown in Figure 2.4. The first step is its hydroxylation at the C_4 position to form *trans*-4-hydroxycyclohexane, which is dehydrogenated to form 4-oxocyclohexane carboxylic acid. Although the precise mechanism is not fully understood, this species is somehow oxidized to form *p*-hydroxybenzoate. The final step is ring cleavage via the *meta* pathway (Trudgill 1984).

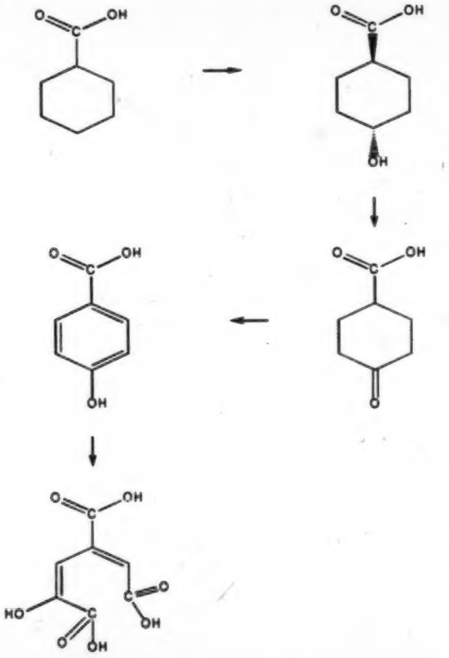

Figure 2.4: Biodegradation Pathway of Cyclohexane Carboxylic Acid

The biodegradation of aromatic hydrocarbons by prokaryotic microorganisms, shown in Figure 2.5, proceeds by oxidation to form a *cis*-dihydrodiol. The *cis*-dihydrodiol is then dehydrogenated so that the benzene nucleus is rearomatized to form a dihydroxylated intermediate (Atlas 1981). This intermediate is then oxidized further either via the *ortho* pathway or via the *meta* pathway. The *ortho* pathway involves cleavage of the bond between the two carbon atoms with the hydroxyl groups, while the *meta* pathway involves the cleavage of the bond between one carbon atom with a hydroxyl group and the adjacent carbon atom without a hydroxyl group (Cerniglia 1984). By contrast, oxidation of aromatic hydrocarbons by eukaryotic microorganisms leads to the formation of a *trans*-dihydrodiol and various other species, such as phenolics and epoxides (Atlas 1981). It should be noted that the biodegradation rate of aromatic hydrocarbons decreases as the number of aromatized rings increases and generally as substitution increases (Cerniglia 1984; Cerniglia 1992).

A biodegradation pathway specific to anthracene by certain *Pseudomonas* species has been proposed, and is shown in Figure 2.6 (Evans *et al.* 1965). It appears that anthracene is oxidized to form *cis*-1,2-dihydroxy-1,2-dihydroanthracene (Gibson and Subramanian 1984). This intermediate is further oxidized to give 1,2-dihydroxyanthracene, which undergoes *meta* cleavage to form *cis*-4-(2-hydroxynaphth-3-yl)2-oxo-but-3-enoic acid (Evans *et al.* 1965). This compound is then attacked on the side of the keto acid to give 2-hydroxy-3-naphthaldehyde, which is dehydrogenated to give 2-hydroxy-3-naphthoic acid (Evans *et al.* 1965). Further biodegradation of this species is not well understood (Gibson and Subramanian 1984).

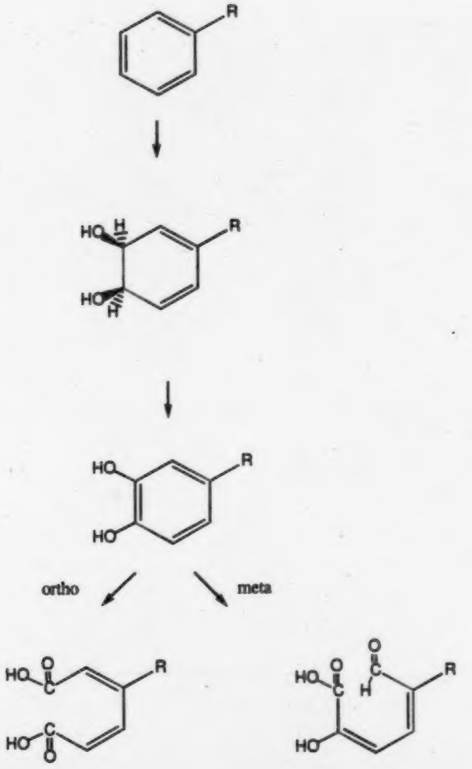

Figure 2.5: Biodegradation Pathway of Aromatic Hydrocarbons

2.1.6 Optimization of Biodegradation

There are many factors that influence the rate of biodegradation. Soil conditions, such as pH, temperature, moisture content, and soil type will have an effect on the activity of the microorganisms (Cerniglia 1992; Loehr 1992; Providenti *et al.* 1993). A lack of inorganic nutrients, such as nitrogen and phosphorus, may also limit biodegradation efforts (Loehr 1992; Efroymson and Alexander 1994; Manilal and Alexander 1991). Water and inorganic nutrients can easily be introduced to the soil if necessary. The pH can also be adjusted if

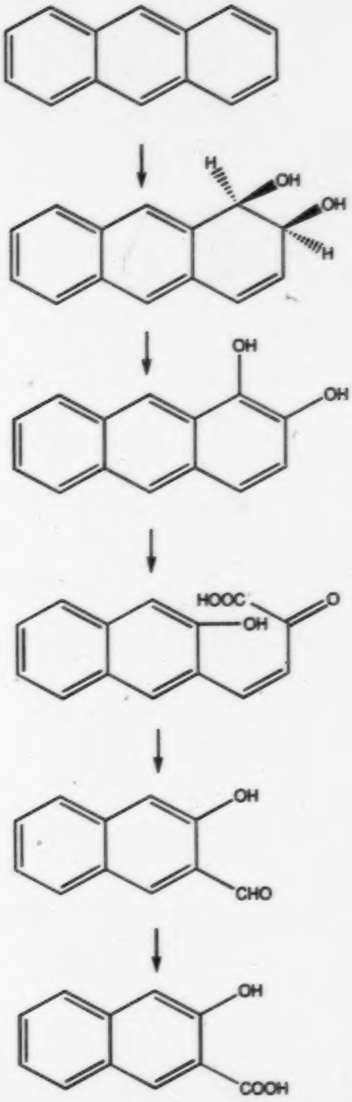
H
OH
OH
H
OH
OH
HOOC
O
OH
OH
CHO
OH
COOH

Figure 2.6: Biodegradation Pathway of Anthracene

the biodegradation site is not too large. Temperature is difficult to control due to seasonal changes, but temporary structures may be used so that biodegradation can occur year round (Providenti *et al.* 1993).

The above conditions also influence the state of hydrophobic contaminants. For example, inadequate soil moisture content will decrease any movement of the contaminant through soil. Sorption of the contaminant onto soil depends on the soil type, pH, and temperature (Providenti *et al.* 1993). A decrease in temperature, for example, increases the viscosity and decreases the volatility of hydrocarbons (Atlas 1991). Highly viscous hydrocarbons tend to sorb to soil particles, which impedes the flow of water through the soil matrix (Morgan and Watkinson 1989).

Oxygen may be the limiting factor for biodegradation (Providenti *et al.* 1993; Heitzer and Sayler 1993). It acts as both a terminal electron acceptor in aerobic respiration and as a substrate in most biodegradation reactions. Although biodegradation of some compounds may proceed under either anaerobic or denitrifying conditions, it proceeds much more efficiently under aerobic conditions because microbial growth is most efficient under aerobic conditions (Mihelcic and Luthy 1988). Thus, efforts should be made to ensure that adequate quantities of oxygen are available. One method of improving aeration is through the use of rotating bioreactors (Gray *et al.* 1994).

In order to attain maximum biodegradation capability, the microbial culture must also be optimized. The cell walls of many Gram positive microorganisms such as *Corynebacteria*, *Mycobacteria*, *Nocardia*, and *Rhodococcus* contain mycolic acids, nocardols, and nocardones. These components make the cell surface extremely hydrophobic, which means that contact with hydrophobic contaminants is more likely to occur (Stephens and Dalton 1987). As a result, these microorganisms may be able to degrade hydrophobic contaminants more effectively than Gram negative microorganisms.

It has also been suggested that the presence of plasmids improves the biodegradation rates of polycyclic aromatic hydrocarbons (Guerin and Jones 1988).

Microorganisms that use various hydrocarbons as their sole carbon source have been isolated. These include *Acinetobacter* species (Rosenberg *et al.* 1980; Breuil and Kushner 1980; Foght *et al.* 1989), *Arthrobacter* species (Efroymson and Alexander 1991; Aamand *et al.* 1995), *Corynebacterium* species (Stephens and Dalton 1987), *Flavobacterium* species (Trzesicka-Mlynarz and Ward 1995), *Mycobacterium* species (Stephens and Dalton 1987; Guerin and Jones 1988; Tiehm 1994), *Nocardia* species (Stephens and Dalton 1987), *Pseudomonas* species (Foght *et al.* 1989; Volkering *et al.* 1992; Volkering *et al.* 1993; Köhler *et al.* 1994; Aamand *et al.* 1995; Bouchez *et al.* 1995; Churchill *et al.* 1995; Grimberg and Aitken 1995; Trzesicka-Mlynarz and Ward 1995), and *Rhodococcus* species (Walter *et al.* 1991; Malachowsky *et al.* 1994; Bouchez *et al.* 1995; Tongpim and Pickard 1996). Many of these isolates, however, will preferentially use other carbon sources, particularly those in the dissolved state. While many microorganisms can adapt to degrading hydrophobic substances after having been grown in the presence of a dissolved carbon source, many other microorganisms lose their ability to degrade hydrophobic substances after having been exposed to a medium free of such substances (Aamand *et al.* 1995). Thus, in order for microorganisms to effectively biodegrade hydrocarbons, efforts should be made to increase the aqueous solubilities of these hydrophobic substances.

2.2 SURFACTANTS

Hydrocarbons are virtually insoluble in water and microorganisms preferentially biodegrade them in the aqueous phase. As a result, bioavailability is a limiting factor in the biodegradation process (Gauger *et al.* 1990). The use of surfactants has been proposed as a means to enhance biodegradation by improving the bioavailability of hydrophobic substances. There have been many investigations into the effects of surfactants on biodegradation, however the conclusions of such studies have varied markedly.

2.2.1 Types of Surfactants

Surface active agents, called surfactants, have a unique chemical structure that consists of both a component that has no attraction for the solvent and a component that has a strong attraction for the solvent. If the solvent is water, the component that has no attraction for water is referred to as the hydrophobic tail, while the component that is strongly attracted to water is referred to as the hydrophilic head. It is the nature of the head group that determines the type of surfactant. The four types of surfactant are nonionic, anionic, cationic, and amphoteric.

Nonionic surfactants do not carry a charge. Instead, their head groups are highly polar. These include polyoxyethylene groups, polyglycerol groups, and polyalkylene oxide copolymers (Aboul-Kassim and Simoneit 1993). These surfactants are used in many industries, such as textiles, detergents, agriculture, paper, cosmetics, and pharmaceuticals (Myers 1988).

The heads of anionic surfactants carry a negative charge. Industrially, these surfactants are used more than any other type (Myers 1988). These surfactants include the alkali carboxylates, sulfates, and sulfonates (Aboul-Kassim and Simoneit 1993). They are used in soaps, shampoos, dishwashing detergents, photographic agents, and corrosion inhibitors (Myers 1988).

The heads of cationic surfactants carry a positive charge. They include the amine salts, quaternary ammonium compounds, and amine oxides (Aboul-Kassim and Simoneit 1993). These surfactants are often potent biocides and act as antiseptic agents in cosmetics, and as fungicides and germicides (Myers 1988).

The heads of amphoteric surfactants contain both positively and negatively charged functional groups. These include imidazoline derivatives, betaines, sulfobetaines, and phosphatides (Aboul-Kassim and Simoneit 1993). They are used in shampoos, textile processing, dry-cleaning fluids, paints, inks, and cosmetics (Myers 1988).

2.2.2 Action of Surfactants

If a system has two or more immiscible phases, an interface must be present. The interface is usually several molecules thick, and the physical properties of these molecules differ from those of the rest of the material. Thus, the interface determines the characteristics and behavior of the entire system. The viability of many applications, including biodegradation, depends on an ability to control and manipulate interfacial interactions (Myers 1988).

The molecules located at an interface have a higher potential energy than those in the rest of the material. These molecules experience a net force field because they interact differently with the molecules closest to them than those in the rest of the material. Surface molecules also interact more strongly with the molecules in the rest of the material than those in the adjacent phase. Surfactants concentrate at available interfaces when they are present at low concentrations because less work is required to transport a surfactant molecule to a surface than any other molecule in the bulk of a material. In doing so, they replace the higher energy bulk phase molecules, which results in a net reduction in the free energy of the entire system (Myers 1988). The work per unit area required to form that new interface is the surface tension of the system, usually reported in units of millinewtons per meter (mN/m) or dynes per centimeter.

An electrical potential also exists across an interface. Charge effects are predominant in aqueous suspensions, dispersions, emulsions, foams, and aerosols. The presence of electrical charges at interfaces is a major determinant of the overall stability of a system (Myers 1988)

The interfacial energy and electrical potential of a system are determined by thermodynamic quantities such as temperature and pressure, and by the chemical composition of the different phases. The interfacial energy is affected by changes in phase compositions, while electrical potential is affected by the addition of ionic materials or by changes in the system pH. The addition of a substance to a system alters the surface tension if the presence of the substance decreases the net free energy of the system. It should be noted that a substance may either raise or lower the surface tension at the interface, although the latter is normally observed (Myers 1988).

2.2.3 The Critical Micelle Concentration

Even after all available interfaces are saturated with surfactant molecules, the overall energy of a system continues to decrease when more surfactant is added. This occurs by the formation of molecular aggregates or micelles that remain in solution. Micelles are formed as the hydrophobic tails of the surfactant molecules come together to create a hydrocarbon pseudophase with a hydrophilic exterior (Rouse *et al.* 1994). These structures act as thermodynamically stable dispersed species that have different properties than individual surfactant molecules in solution. As more and more surfactant is dissolved in water, the energy change it experiences forming micelles is less than the energy change it would experience in becoming an individual molecule in solution. As a result, the formation of micelles favors an increase in solubility. As the concentration of surfactant in aqueous solution changes, the concentration of individual surfactant molecules in solution may increase or decrease slightly, but micelles will be the predominant form of surfactant above a certain concentration of surfactant. This concentration is called the critical micelle concentration, or CMC (Myers 1988).

The addition of a surfactant at a concentration higher than the CMC to a system containing a hydrophobic substance leads to the formation of a thermodynamically stable, isotropic solution of this substance (Myers 1988). This phenomenon is referred to as solubilization. The mechanism of solubilization involves the diffusion of micelles to the interface between the hydrophobic substance and the water. At the interface, the micelles dissociate into surfactant monomers and adsorb the hydrophobic material. The monomers then desorb from the interface as they reaggregate into micelles, with an additional amount of hydrophobic material in the core of the micelle (Rosen 1989).

The equilibrium of a hydrophobic compound between micelle and aqueous phase can be described by a partition coefficient, K_m, which is defined as (Jafvert 1991; Edwards *et al.* 1991):

$$K_m = \frac{C_{mic}}{C_{aq}}$$

where C_{mic} is the concentration of the hydrophobic material in the micelle and C_{aq} is the concentration of the hydrophobic material in the aqueous phase.

2.2.4 Factors that Affect the CMC

There are many factors that affect the critical micelle concentration of a surfactant. The structure of a surfactant, for example, is one determining factor. In general, the CMC in aqueous solution decreases as the number of carbon atoms in the hydrophobic group increases. Similarly, the closer the hydrophilic group is to a terminal position, the lower the CMC (Rosen 1989). It should be noted that factors that decrease the CMC also decrease the likelihood that the surfactant will precipitate (West and Harwell 1992).

The presence of electrolytes also affects the CMC. For anionic and cationic surfactants, the CMC decreases because the thickness of the ionic atmosphere surrounding the ionic head groups decreases in the presence of the additional electrolyte. This results in a decrease in electrical repulsion between surfactant molecules in the micelle. For nonionic and amphoteric surfactants, the CMC changes as a result of the salting out or salting in of the hydrophobic groups in the water by the electrolyte (Rosen 1989).

The presence of organic materials may also affect the CMC. Polar organic materials may decrease the CMC by being incorporated into the micelle. Other organic materials, such as urea, formamide, guanidinium salts, short-chain alcohols, water-soluble esters, and polyhydric alcohols such as fructose and xylose, also affect the CMC by modifying water-micelle interactions. Urea, formamide, and guanidinium salts increase the CMC of surfactants because they disrupt the water structure, causing an increase in the degree of hydration of the hydrophilic group, which inhibits the formation of micelles. By contrast, xylose and fructose decrease the CMC because they promote water structure by decreasing the degree of hydration of the hydrophilic group, which enhances the formation of micelles. Short-chain alcohols and water-soluble esters increase the CMC by decreasing the solubility parameter of the water. This increases the solubility of the monomeric form of the surfactant, which in turn, increases the CMC (Rosen 1989).

The presence of a second liquid phase may also affect the CMC. If the second liquid phase is polar, the CMC decreases because this second liquid adsorbs in the outer portion of the surfactant micelle. Conversely, if the second liquid phase is not very polar, the CMC increases because this second liquid dissolves in water, increasing its solubility parameter (Rosen 1989).

Temperature also affects the CMC of surfactants, but the effects are complex. As temperature increases, the CMC decreases to some minimum. Once this minimum is attained, the CMC increases as the temperature continues to increase. An increase in temperature causes decreased hydration of the hydrophilic group, which enhances the formation of micelles. However, an increase in temperature also causes disruption of the water structure that surrounds the hydrophobic group, which inhibits the formation of micelles. The relative magnitude of these opposing effects determines whether the CMC increases or decreases within a temperature range (Rosen 1989).

2.2.5 Surfactants and Soil Clean-Up

The cleaning of a solid substrate, such as soil, involves the removal of unwanted foreign material from its surface. Detergent action is promoted by the interactions of the hydrophobic portion of the surfactant molecule with both the unwanted material and the soil. Adsorption alters the chemical, electrical, and mechanical properties of the various interfaces and depends strongly on the nature of each component. Since most soils are negatively charged in water, the addition of cationic surfactants can have a detrimental effect on detergency because the surfactant tends to sorb to the soil particles (Myers 1988). Anionic surfactants, however, do not tend to sorb to soil, but they are most likely to precipitate (West and Harwell 1992). Nonionic surfactants are generally more effective solubilizers than either anionic or cationic surfactants (Saito and Shinoda 1967).

Three important criteria for the selection of surfactants for soil clean-up are cleaning efficiency, economic viability, and environmental behavior (Hurtig *et al.* 1988). The cleaning efficiency of a surfactant is determined by the capacity of the surfactant to mobilize the hydrophobic material to be removed. The economic viability of a surfactant is determined by the price, the concentration to be used, and the possibility of its recovery and reuse. Surfactant recovery has been described in the literature (Gannon *et al.* 1989; Clarke *et al.* 1991). The environmental criterion is that the surfactant itself must be readily biodegradable or easily removed from the system (Hurtig *et al.* 1988).

There are several ways in which hydrophobic materials may be mobilized in the presence of surfactants. The presence of micelles, for example, allows hydrophobic materials to be dissolved in solvents in which they are normally insoluble. The presence of surfactant molecules at the interface between two immiscible phases reduces the surface tensions of these phases, which allows the molecules in each phase to cross the boundary between them more easily. The decrease in surface tension may also lead to an enhancement in the water wettability of surfaces by allowing water to displace the molecules of one phase at the surface of another.

Some parameters that determine the ability of a surfactant to mobilize hydrocarbons include surface tension minimization, CMC, and solubilization efficiency. Each of these parameters is probably useful to some degree, but by itself inadequate (Vigon and Rubin 1989).

Surfactants in soil may hydrolyze to form flocs, combine to form micelles, disperse soil colloids, or form viscous emulsions with petroleum products. Any of these processes can lead to clogging of the soil pores, which decreases the flow of aqueous material through the soil matrix (Abdul *et al.* 1990).

Biodegradation may also be affected if the surfactant itself adheres to soil. This may result in the surfactant being unavailable for hydrocarbon solubilization. Other effects include a retardation of the transport of surfactant through soil, a retardation of the transport of hydrophobic materials through soil, or a reduction in the availability of surfactant for biodegradation (Liu *et al.* 1992). This should be considered in the selection of surfactants.

2.3 USE OF SURFACTANTS IN SOIL BIODEGRADATION

Because of their ability to mobilize hydrophobic substances, the use of surfactants has been identified as a possible means to increase the biodegradation rates of such materials. A close examination of the actual effects of surfactants on biodegradation efforts, however, suggests that this may not be true.

2.3.1 Solubility of Organic Material in the Presence of Surfactants

Many research efforts have focused on the effects of both hydrocarbon composition and surfactant type on the ability of the surfactant to solubilize organic materials. It is clear from these investigations that regardless of the hydrophobic material or type of surfactant, the aqueous solubility of the hydrophobic material will increase in the presence of surfactants.

Studies have been conducted to determine whether or not various nonionic surfactants increase the solubility of anthracene (Liu *et al.* 1991), benzene (Diallo *et al.* 1994), biphenyl (Abdul and Gibson 1991; Abdul *et al.* 1992), chlorinated hydrocarbons (Rickabaugh *et al.* 1986), cyclohexane (Diallo *et al.* 1994), decane (Diallo *et al.* 1994), *o*-dichlorobenzene (Diallo *et al.* 1994), dodecane (Pennell *et al.* 1993; Diallo *et al.* 1994), hexachlorobenzene (Jafvert *et al.* 1994), hexane (Diallo *et al.* 1994), naphthalene (Edwards *et al.* 1990; Edwards *et al.* 1991; Edwards *et al.* 1992b; Strong-Gunderson and Palumbo 1995), phenanthrene (Grimberg *et al.* 1994; Edwards *et al.* 1990; Edwards *et al.* 1991; Edwards *et al.* 1992a; Edwards *et al.* 1992b; Edwards *et al.* 1994a; Liu *et al.*

1991), pyrene (Edwards *et al.* 1990; Edwards *et al.* 1991; Edwards *et al.* 1992a; Edwards *et al.* 1992b; Liu *et al.* 1991), tetrachloroethylene (Fountain *et al.* 1991; Diallo *et al.* 1994), toluene (Diallo *et al.* 1994; Strong-Gunderson and Palumbo 1995), transmission fluid (Abdul *et al.* 1990), trichloroethylene (Diallo *et al.* 1994), and *o*-xylene (Diallo *et al.* 1994). In all cases, the presence of surfactants increased the solubility of the various hydrophobic materials.

Some studies have also been conducted to determine the effects of various anionic surfactants on the solubility of anthracene (Liu *et al.* 1991; Roy *et al.* 1994), biphenyl (Gannon *et al.* 1989; Clarke *et al.* 1991), carbon tetrachloride (Park and Jaffé 1993), chlorinated hydrocarbons (Rickabaugh *et al.* 1986), dichlorobenzene (Gannon *et al.* 1989), hexachlorobenzene (Jafvert et. al 1994), hexadecane (Thangamani and Shreve 1993), naphthalene (Park and Jaffé 1993; Gannon *et al.* 1989), phenanthrene (Jafvert 1991; Park and Jaffé 1993; Liu *et al.* 1991, pyrene (Liu *et al.* 1991; Jafvert 1991), and transmission fluid (Abdul *et al.* 1990). As before, the presence of surfactants increased the solubility of all of these materials.

A few studies have also been conducted to determine the effects of cationic surfactants on chlorinated hydrocarbons (Rickabaugh *et al.* 1986; Wagner *et al.* 1994), DDT (Kile and Chiou 1989), and trichlorobenzene (Kile and Chiou 1989). The presence of cationic surfactants also increases the solubility of these materials.

2.3.2 Effects of Surfactants on Biodegradation

While there is no doubt that surfactants enhance the solubility of hydrophobic compounds, there is some dispute about whether or not surfactants enhance biodegradation. Table 2.2 shows a partial list of some of the work that has been done in the field, along with the conclusion about the effect of surfactants on biodegradation.

Many explanations for the apparent enhancement of biodegradation in the presence of surfactants have been proposed. For example, by increasing the solubility of hydrophobic substances, it is widely assumed that these substances become more accessible to microorganisms, and that this automatically means that the microorganisms will biodegrade these materials more effectively (Rittman and Johnson 1989; Breuil and Kushner 1980; Robichaux and Myrick 1972; Aronstein and Alexander 1992; Liu *et al.* 1995; Volkering *et al.* 1995b). In reality, however, this is not always true.

It is also possible that biodegradation is enhanced because the presence of surfactant prevents reaggregation of contaminant crystals in the case of solid polycyclic aromatic hydrocarbons (Köhler *et al.* 1994). Other explanations are that the solubilization effect of the surfactant reduces the time required for the cell culture to double (Bury and Miller 1993) and that the surfactant causes a change in cell wall properties so that the hydrophobic substance can enter the cell more readily (Breuil and Kushner 1980). There is also some evidence that low agitation enhances biodegradation in the presence of surfactants because agitation increases the surface area by dispersing the hydrophobic substance (Fu and Alexander 1995; Köhler *et al.* 1994).

Table 2.2: Studies Reporting the Apparent Effects of Surfactants on Biodegradation

Reference	Type of Surfactant	Surfactant Concentration	Type of Microorganism	Organic Contaminant	Effect on Biodegradation
Aiba *et al.* 1969	Nonionics	< CMC	Yeast	*n*-Alkanes	Inhibited
Aronstein *et al.* 1991	Nonionics	< CMC		Phenanthrene and biphenyl	Mixed results
Aronstein and Alexander 1992	Nonionics	< CMC		Phenanthrene and biphenyl	Enhanced
Aronstein and Alexander 1993	Nonionic	< CMC		Phenanthrene and biphenyl	Enhanced
Breuil and Kushner 1980	Nonionics	> CMC	Gram negative	Hexadecane	Enhanced
Bury and Miller 1993	Nonionic	> CMC	Gram negative	Decane and tetradecane	Enhanced
Churchill *et al.* 1995	Nonionics	> CMC	Gram negative	Phenanthrene	Mixed results
Deschênes *et al.* 1995b	Anionic	< CMC	Mixed	PAHs	No effect
Deschênes *et al.* 1995b	Anionic	> CMC	Mixed	PAHs	Inhibited
Efroymson and Alexander 1991	Nonionic	> CMC	Gram positive	Hexadecane	Inhibited
Efroymson and Alexander 1991	Nonionic	> CMC	Gram positive	Naphthalene	Enhanced
Fu and Alexander 1995	Nonionics	> CMC		Phenanthrene and biphenyl	Mixed results
Fu and Alexander 1995	Nonionics	< CMC		Phenanthrene and biphenyl	Mixed results
Fu and Alexander 1995	Anionics	> CMC		Phenanthrene and biphenyl	Mixed results
Fu and Alexander 1995	Anionics	< CMC		Phenanthrene and biphenyl	Mixed results
Ghosh *et al.* 1994	Nonionic	> CMC	Gram negative	Phenanthrene, naphthalene, PCBs	No effect
Ghosh *et al.* 1995	Nonionic	> CMC	Mixed	PAHs	No effect
Grimberg and Aitken 1995	Nonionic	> CMC	Gram negative	Phenanthrene	Inhibited
Guerin and Jones 1988	Nonionics	> CMC	Gram positive	Phenanthrene	Enhanced
Köhler *et al.* 1994	Nonionics	> CMC	Gram negative	Phenanthrene	Enhanced

Reference	Type of Surfactant	Surfactant Concentration	Type of Microorganism	Organic Contaminant	Effect on Biodegradation
Laha and Luthy 1991	Nonionics	> CMC	Mixed	Phenanthrene	Inhibited
Laha and Luthy 1991	Nonionics	< CMC	Mixed	Phenanthrene	No effect
Laha and Luthy 1992	Nonionics	> CMC	Mixed	Phenanthrene	Inhibited
Lantz *et al*. 1995	Nonionic	> CMC	Gram negative	Fluoranthene	Enhanced
Liu *et al*. 1995	Nonionics	> CMC	Mixed	Naphthalene	Enhanced
Liu *et al*. 1995	Nonionics	< CMC	Mixed	Naphthalene	No effect
Mulkins-Philips and Stewart 1974	Dispersants		Mixed	Crude oil	Mixed results
Rittman and Johnson 1989	Dispersant		Mixed	Lubricating oil sorbed to soil	No effect
Rittman and Johnson 1989	Dispersant		Mixed	Lubricating oil in liquid phase	Enhanced
Robichaux and Myrick 1972	Dispersants		Mixed	Crude oil	Mixed results
Roch and Alexander 1995	Nonionics	> CMC	Mixed	Phenanthrene and biphenyl	Mixed results
Roch and Alexander 1995	Nonionics	< CMC	Mixed	Phenanthrene and biphenyl	Mixed results
Roch and Alexander 1995	Anionics	> CMC	Mixed	Phenanthrene and biphenyl	Mixed results
Roch and Alexander 1995	Anionics	< CMC	Mixed	Phenanthrene and biphenyl	Mixed results
Tiehm 1994	Nonionics	> CMC	Gram positive	PAHs	Inhibited
Tiehm 1994	Anionic	> CMC	Mixed	Phenanthrene	Inhibited
Tiehm 1994	Nonionics	> CMC	Mixed	PAHs	Mixed results
Viney and Bewley 1990	Nonionics	> CMC	Mixed	PCBs	Inhibited
Volkering *et al*. 1995a	Nonionics	> CMC	Gram negative	Phenanthrene and naphthalene	Enhanced
Volkering *et al*. 1995b	Nonionics	> CMC	Gram negative	Phenanthrene and biphenyl	Enhanced
Volkering *et al*. 1995b	Nonionics	< CMC	Gram negative	Phenanthrene and biphenyl	No effect
Wilson *et al*. 1995	Nonionics	> CMC	Mixed	Petroleum hydrocarbons	No effect
Wilson *et al*. 1995	Anionics	> CMC	Mixed	Petroleum hydrocarbons	Inhibited
You *et al*. 1995	Nonionic	> CMC		DDT	Enhanced

There are also several reasons that have been proposed to explain the apparent inhibition of biodegradation in the presence of surfactants in a number of studies. For example, surfactants may simply prevent microorganisms from adhering to hydrophobic substances (Aiba *et al.* 1969). It is also possible that surfactants are toxic to microorganisms (Laha and Luthy 1991), the contaminant in the micelle is unavailable to microorganisms (Laha and Luthy 1991; Laha and Luthy 1992; Grimberg and Aitken 1995; Volkering *et al.* 1995a), or surfactants act as a preferential substrate for the microorganisms (Laha and Luthy 1991; Tiehm 1994; Deschênes *et al.* 1995b). Biodegradation may be inhibited because partitioning of hydrophobic substances from the nonaqueous phase to the aqueous phase is slow in the presence of surfactants (Fu and Alexander 1995) or because surfactants themselves sorb onto soil (Laha and Luthy 1991; Laha and Luthy 1992). It has also been suggested that surfactants alter microbial membranes and proteins (Laha and Luthy 1992) or that surfactants alter microbial metabolism in some other way (Roch and Alexander 1995; Laouar *et al.* 1996) or that surfactants induce a deficiency in some inorganic nutrient that is essential for microbial growth (Fu and Alexander 1995).

The toxicity of surfactants is one aspect of surfactant-microorganism interactions that is currently being debated in the literature. One view is that surfactants are toxic to microorganisms (Viney and Bewley 1990; Laha and Luthy 1991; Tiehm 1994; Fu and Alexander 1995), while the opposing view is that surfactants are not toxic to microorganisms (Liu *et al.* 1995). It has been suggested that the presence of surfactants inhibits microbial growth (Schlictman *et al.* 1995; Laouar *et al.* 1996). Conversely, there is also a suggestion that surfactants are essential for microbial growth on hydrophobic substrates because the presence of surfactants prevents the formation of conglomerates of hydrophobic substances (Rittman and Johnson 1989) and because the presence of micelles tends to allow ionic nutrients to concentrate near the surface of micelles (Velankar *et al.* 1975). It is quite likely that the effect of a given surfactant on the growth of a certain microorganism depends on the type of microorganism (Cserháti *et al.* 1991). It should be noted that the inhibition of microbial growth in the presence of surfactants is not necessarily the result of toxicity.

There have been several interesting observations concerning surfactant-microorganism interactions. In one case, for example, of several dispersants tested, the poorest emulsifier was the only one to actually stimulate biodegradation (Mulkins-Philips and Stewart 1974). Surfactant blends appear to be more effective for biodegradation (Rickabaugh *et al.* 1986; Saito and Shinoda 1967) because mixtures of nonionics and ionics tend to have a lower CMC, which decreases precipitation of the surfactant (West and Harwell 1992). Biodegradation may also be more effective at surfactant concentrations below the CMC than above the CMC, even if the biodegradation rates under both sets of conditions are improved, possibly because some of the contaminant may be inside the surfactant micelle and unavailable to the microorganisms (Aronstein and Alexander 1993). It has been noted that surfactants may only increase the initial rate of biodegradation, while the total amount of hydrocarbon ultimately biodegraded is the same whether surfactants were present or not (Lantz *et al.* 1995). Finally, it appears that mixed cultures biodegrade hydrocarbons more effectively than isolated cultures (Tiehm 1994).

Clearly, the efficiency of biodegradation depends on many factors. There is no question that the type of microorganisms present is a key determinant because of the way each microorganism reacts to a certain surfactant (Churchill *et al.* 1995; Fu and Alexander 1995). However, the factors that determine these interactions are not well defined. This discussion indicates that the mechanism of biodegradation in the presence of surfactants is poorly understood.

2.3.3 Use of Biosurfactants

Biosurfactants have also been tested for their ability to promote biodegradation. An advantage of these surfactants is that they are probably less toxic to microorganisms than chemical surfactants because they are produced by the microorganisms themselves. Another advantage of biosurfactants is that they may be biodegraded more readily than many commercial surfactants (Van Dyke *et al.* 1993).

There are many different types of biosurfactants, but the main groups are glycolipids, emulsan, and surfactin (Parkinson 1985). Different types of glycolipids are trehalose dimycolates, trehalose di-corynemycolates, rhamnolipids, and sophorose lipids. Source organisms for glycolipids include *Mycobacteria* species, *Nocardia* species, *Rhodococcus* species, *Corynebacteria* species, *Pseudomonas* species, and *Torulopsis* (*Candida*) species (Parkinson 1985). Microorganisms that produce emulsan include *Acinetobacter* species, while microorganisms that produce surfactin include *Bacillus* species (Parkinson 1985).

Like commercial surfactants, biosurfactants increase the solubilities of various hydrocarbons (Van Dyke *et al.* 1993; Scheibenbogen *et al.* 1994). However, as was the case for commercial surfactants, there is some dispute about the effects of biosurfactants on biodegradation. Table 2.3 shows a list of some studies that have been conducted to determine the effects of certain biosurfactants on biodegradation.

The general consensus is that biosurfactants have more potential for biodegradation than chemical surfactants (Scheibenbogen *et al.* 1994; Deschênes *et al.* 1995a; Ghosh *et al.* 1995), even though the conclusions about the effect of biosurfactants on biodegradation vary. Biosurfactant production seems to be enhanced if dissolved hydrocarbons are present in the growth medium (Parkinson 1985), and there is some thought that biosurfactants allows better contact between the cells and the organic substrate (Breuil and Kushner 1980). It has been observed, however, that biosurfactant effectiveness is inversely proportional to the solubilities of the hydrocarbons to be biodegraded (Jain *et al.* 1992). Other key factors of biosurfactant effectiveness are the type of microorganism involved (Zhang and Miller 1995) and the type of soil involved (Providenti *et al.* 1995).

2.4 THE ROLE OF ADHESION IN BIODEGRADATION

It is clear that improving the effectiveness of biodegradation in the aqueous phase is not by itself sufficient to reduce the concentrations of many hydrophobic contaminants to acceptable levels. Thus, methods that improve the effectiveness of biodegradation in the presence of a nonaqueous phase should be examined. This would involve promoting the movement of the microorganisms from the aqueous phase to the nonaqueous phase so that they may adhere to the surfaces of the hydrophobic contaminants. The first step in this process is to identify the factors which favor microbial adhesion to such surfaces.

Table 2.3: Studies Reporting the Apparent Effects of Biosurfactants on Biodegradation

Reference	Type of Biosurfactant	Type of Microorganism	Organic Contaminant	Effect on Biodegradation
Breuil and Kushner 1980	Lipids	Gram negative	Hexadecane	Enhanced
Breuil and Kushner 1980	Long chain fatty acids	Gram negative	Hexadecane	Enhanced
Breuil and Kushner 1980	Short chain fatty acids	Gram negative	Hexadecane	Inhibited
Deschênes *et al.* 1995a	Rhamnolipid	Mixed	PAHs	Inhibited
Falatko and Novak 1992	Biosurfactants grown on glucose and vegetable oil	Mixed	Gasoline	Inhibited
Falatko and Novak 1992	Biosurfactants grown on gasoline	Mixed	Gasoline	No effect
Foght *et al.* 1989	Emulsan	Mixed	Crude oil alkanes and aromatics	Inhibited
Foght *et al.* 1989	Emulsan	Isolates	Crude oil alkanes	Inhibited
Foght *et al.* 1989	Emulsan	Isolates	Crude oil aromatics	No effect
Gauger et al. 1990	Bioemulsifiers	Mixed	PAHs	Enhanced
Ghosh *et al.* 1994	Rhamnolipid	Gram negative	PCBs	Enhanced
Jain *et al.* 1992	Rhamnolipid	Gram negative	Tetradecane, hexadecene, pristane	Enhanced
Jain *et al.* 1992	Rhamnolipid	Gram negative	2-Methylnaphthalene	No effect
Oberbremer *et al.* 1990	Glycolipids	Mixed	Mixed hydrocarbons	Enhanced
Providenti *et al.* 1995	Rhamnolipids	Gram negative	Phenanthrene	Mixed results
Zhang and Miller 1992	Rhamnolipid	Gram negative	Octadecane	Enhanced
Zhang and Miller 1995	Rhamnolipids	Gram negative	Hexadecane and Octadecane	Mixed results

2.4.1 The Bacterial Cell Wall Structure

The adhesion capability of bacteria is most likely related to the nature of the components in the bacterial cell wall. There are two types of bacteria according to the way they stain in the Gram reaction: Gram positive bacteria and Gram negative bacteria. These two types of bacteria have very different cell wall structures, as shown in Figure 2.7.

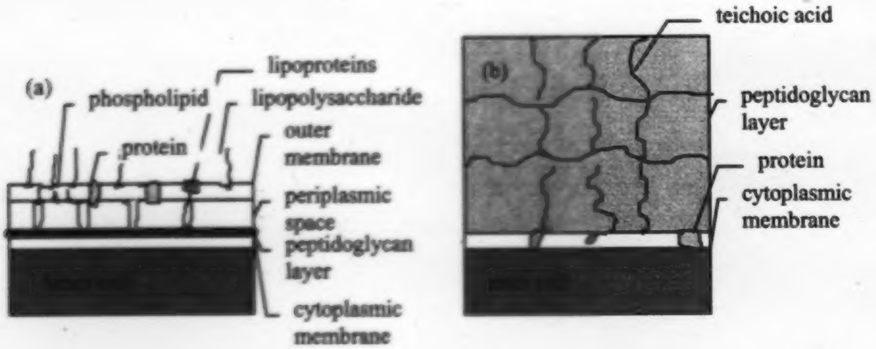
(a)
phospholipid
lipoproteins
lipopolysaccharide
protein
outer membrane
periplasmic space
peptidoglycan layer
cytoplasmic membrane
(b)
teichoic acid
peptidoglycan layer
protein
cytoplasmic membrane

Figure 2.7: Bacterial Cell Wall Structure (a) Gram Negative Bacteria; (b) Gram Positive Bacteria.

The major component of Gram positive cell walls is peptidoglycan, which can comprise anywhere from 50 to 80 percent of the cell wall mass. Peptidoglycan consists of long glycan chains with alternating residues of muramic acid and glucosamine. The carboxyl groups of the muramic acid residues are substituted with short peptide side chains which are cross-linked from one glycan chain to another (Ward and Berkeley 1980). Despite the fact that the walls of Gram positive bacteria also contain several secondary wall polymers, such as teichoic acids, it is mainly peptidoglycan that is exposed at the surface of Gram positive bacteria.

Conversely, peptidoglycan makes up between 8 and 15 percent of the mass of the Gram negative cell wall. The peptidoglycan is in the form of a thin, dense layer that covers the cytoplasmic membrane. To the exterior of the peptidoglycan layer is the periplasmic space, which is a zone that separates the peptidoglycan layer from the outer membrane. The outer membrane contains several proteins, lipids, and polysaccharides, which serve as receptor sites for various molecules (Ward and Berkeley 1980).

Another staining reaction that is used to distinguish types of bacteria is the acid-fast stain. This stain binds strongly only to bacteria that have a waxy material in their cell walls, such as *Mycobacterium* species, *Nocardia* species, and *Rhodococcus* species (Tortora *et al.* 1992). It is this waxy material that may contribute to the hydrophobic nature of such microorganisms.

2.4.2 Microbial Hydrophobicity and Adhesion

The ability of a microorganism to adhere to a certain surface depends on how it interacts with other substances in its environment. Because microorganisms tend to remain in the aqueous phase, their interactions with water are extremely important. The way in which they interact with water is referred to as hydrophobicity. A microorganism that has a high affinity for water has low hydrophobicity, while a microorganism that has a low affinity for water has high hydrophobicity. Microorganisms that have high hydrophobicity tend to remain at interfaces.

The degree of hydrophobicity of each microorganism varies according to the nature and amount of components that promote or reduce hydrophobicity within the microbial cell wall. The components that promote hydrophobicity are called hydrophobins, while the components that reduce hydrophobicity are called hydrophilins. Both hydrophobins and hydrophilins coexist on the surfaces of all bacteria (Rosenberg and Doyle 1990).

The biological significance of hydrophobicity is not clearly understood. For example, it is unclear whether hydrophobic surface properties are important for microbial functions, or if hydrophobic surface properties are simply a measurement that reflect the presence of certain surface components (Rosenberg and Doyle 1990). It is clear, however, that cell surface hydrophobicity can be promoted by the presence of certain proteins with specific amino acid sequences and that only one or two strains within a given species may adhere to a given surface (Rosenberg and Doyle 1990).

Even though adhesion of bacteria to surfaces depends on many factors, hydrophobicity is probably the most important (Dahlbäck *et al.* 1981; van Loosdrecht *et al.* 1987; Bendinger *et al.* 1993; Huysman and Verstraete 1993). If the surface tension of the bacteria is higher than the surface tension of the suspending medium, hydrophilic substances adhere to surfaces better than hydrophobic substances. If the surface tension of the suspending medium is higher than that of the bacteria, the reverse is true (Absolom *et al.* 1983). As a result, in aqueous media, high hydrophobicity coincides with enhanced adhesion (Stenström 1989). There is a definite correlation between the ability of a substance to decrease the surface tension and inhibit adhesion (Whitekettle 1991).

2.4.3 Microbial Growth on Surfaces

The first step in microbial growth is the movement of cells to a particular substrate (van Loosdrecht *et al.* 1990). This may occur by diffusive transport, convective transport, or active movement. Diffusive transport is extremely slow, but it is responsible for transport across interfaces, particularly if no liquid gradient exists. Convective transport is much faster, and is the result of flowing liquid. Active movement is the result of cellular functions that allow microorganisms to propel themselves.

The next step is initial adhesion, which seems to be the rate limiting step in microbial growth (Rosenberg *et al.* 1981; Rosenberg and Rosenberg 1981). This is the result of the Gibbs free energy change that occurs as two nonpolar molecules approach one another in aqueous solution. These molecules are surrounded by structured layers of water. The degree of structure is inversely proportional to the distance between the nonpolar molecules. Water molecules in such layers are unable to undergo hydrogen bonding in all directions. Thus, they are at a higher energy level than the water molecules in the bulk solution. If the nonpolar molecules are incapable of interacting with water molecules, then energy is required to bring these molecules into the water phase (Rosenberg and Doyle 1990). The ensuing breaking of bonds and subsequent formation of new hydrogen bonds leads to a small negative enthalpy change. Thus, the energy input is attributed to the negative entropy change (Rosenberg and Kjelleberg 1986). The free energy is obtained from the summation of the attractive van der Waals forces and the repulsive electrostatic forces (van Loosdrecht *et al.* 1990).

The next step in microbial growth is firm attachment. This occurs as special cell structures such as fibrils and polymers form strong links between the microorganism and the substrate (van Loosdrecht *et al.* 1990). This step may be affected either negatively or positively by the presence of certain substances in the medium.

The final step in microbial growth is colonization. This occurs by the formation of several microcolonies or a single biofilm on the substrate. As the colonies grow, some microorganisms are released into the aqueous medium as a result of shear forces in the environment (van Loosdrecht *et al.* 1990). The microorganisms remain in the aqueous phase until the growth cycle is able to start over again.

2.4.4 Factors that Affect Microbial Adhesion

The extent of adhesion depends on both the availability of the substrate and the affinity of the microorganisms for the substrate. This is determined by the surface properties of the microorganisms, the surface properties of the substrate, and the conditions of the surrounding environment (Absolom *et al.* 1983).

The components of the microbial cell surface vary as a function of growth conditions such as aeration, temperature, growth medium, and age of cells (Rosenberg and Kjelleberg 1986). For example, exponential phase microorganisms are less likely to adhere to surfaces than stationary phase microorganisms (Rosenberg and Rosenberg 1985). Similarly, the presence of supplementary substances such as amino acids (Rosenberg and Rosenberg 1985) and capsular polysaccharides (Rosenberg *et al.* 1983b) in the growth medium may inhibit adhesion. Thus, differences in hydrophobicity may exist not only from species to species, but also within a strain as a result of nutritional status.

Charge may also play a role in microbial adhesion. When a negatively charged microorganism approaches a negatively charged substrate, there is a tendency for a mutual repulsion. However, van der Waals attraction energies are able to counteract the repulsion energies. In fact, it has been found that the negative charges on the microorganism cell surface do not play any role in adhesion, although the positive charges do (Stenström 1989).

The movement of cells through soil may also be an important factor for biodegradation. Charge, hydrophobicity, and the presence of capsules or flagella are important in determining whether a microorganism will be able to move through a soil matrix, however there does not appear to be a correlation between these parameters and the ability of a microorganism to move through such a matrix (Gannon *et al.* 1991). The size of the microorganisms seems to be the most important physicochemical parameter that determines whether they can be moved easily, as smaller microorganisms are moved much more readily than larger ones (Gannon *et al.* 1991).

The nature of the mode of transport also seems to be important in microbial adhesion. Adhesion of microorganisms to surfaces is more efficient in dynamic systems where transport is dominated by both convection and diffusion than in static systems where transport is by diffusion only (Rijnaarts *et al.* 1993).

Finally, adhesion seems to be determined by the state of the hydrocarbons, and not the ability of the bacteria to grow on a given hydrocarbon. Hydrocarbons are utilized more readily in the liquid phase than in the solid phase (Reddy *et al.* 1982). Further, a microorganism may not adhere to a given hydrocarbon even if it is capable of degrading other hydrocarbons, while microorganisms that are not capable of degrading any hydrocarbons may adhere in high proportion to certain hydrocarbons (Rosenberg *et al.* 1980).

2.4.5 Effect of Microbial Adhesion on Biodegradation

Several studies have been conducted in order to identify microbial strains that are capable of promoting the biodegradation of certain hydrophobic contaminants by first adhering to their surfaces. Many microorganisms, however, will not adhere to surfaces, and will biodegrade contaminants in the liquid phase only. For example, it has been found that certain *Pseudomonas* species will degrade biphenyl (Wodzinski and Bertolini 1972), naphthalene (Wodzinski and Bertolini 1972), hexadecane (Rosenberg and Rosenberg 1985), octane (Rosenberg and Rosenberg 1985), phenanthrene (Wodzinski and Coyle 1974), and xylene (Rosenberg and Rosenberg 1985) in the aqueous phase only.

Some microorganisms have been found to be able to biodegrade liquid contaminants after adhering to their surfaces. Several *Acinetobacter* species are able to grow on hexadecane (Rosenberg and Rosenberg 1981; Rosenberg and Rosenberg 1985), and those species that adhered to the surface better than the others were able to grow faster than the others (Rosenberg and Rosenberg 1985). It has also been found that *Arthrobacter* species are able to biodegrade naphthalene and hexadecane dissolved in 2,2,4,4,6,8,8-heptamethylnonane after adhering to the water-heptamethylnonane interface.

Few microorganisms that are able to adhere to solid contaminants have been identified. However, the growth of a *Rhodococcus* species on anthracene has been observed (Tongpim and Pickard 1996).

2.4.6 Effect of Surfactants on Microbial Adhesion to Hydrocarbons

Since microbial adhesion has been identified as the first step in the biodegradation process, and surfactants have been identified as a means to increase bioavailability, the effect of surfactants on microbial adhesion should be studied.

The presence of nonionic surfactants appears to inhibit the adhesion of microorganisms to hydrophobic surfaces, and is indicated by a decline in microbial growth (Whitekettle 1991; Efroymson and Alexander 1991; Ortega-Calvo and Alexander 1994).

The presence of the biosurfactant emulsan also inhibits the adhesion of microorganisms to hydrophobic surfaces (Rosenberg *et al.* 1983a; Pines and Gutnick 1984b) and desorbs previously bound microorganisms from hydrophobic surfaces (Rosenberg *et al.* 1983a). It appears that emulsan takes over the function of receptor by assuming a specific conformation that allows it to interact accordingly with hydrophobic surfaces (Pines and Gutnick 1984b).

The presence of rhamnolipid enhances the adhesion of microorganisms with low cell hydrophobicity, but it has no effect on the adhesion of microorganisms with high cell hydrophobicity (Zhang and Miller 1994). The result of this is that the presence of rhamnolipid enhances biodegradation by microorganisms that have low cell hydrophobicity, and it inhibits biodegradation by microorganisms that have high cell hydrophobicity.

Since many factors are involved in the biodegradation process, a mechanism that explains the interactions between microorganisms, hydrocarbons, and surfactant should be identified.

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Soil Extracts

Organic extracts from four different contaminated soils were used. The soils originated from industrial sites in Edmonton, Alberta; Prince Albert, Saskatchewan; Devon, Alberta; and Montreal, Quebec. The Edmonton (EDM) and Prince Albert (PAA) soils were primarily contaminated with creosote, while the Devon (DEV) and Montreal (MTL) soils were primarily contaminated with petroleum hydrocarbons. The Montreal soil had been partially bioremediated before the commencement of the investigation.

3.1.2 Microorganisms

Two microorganisms were used. One was identified as a Gram positive *Rhodococcus* species (Tongpim and Pickard 1996), and the other was a Gram negative *Pseudomonas* species (Gray *et al.* 1994). Both species were isolated from a soil population enriched for growth on anthracene, and were stored in glycerol at -20°C.

The microbial growth medium contained 1.33 g/L KH_2PO_4, 2.67 g/L K_2HPO_4, 1 g/L NH_4Cl, 2 g/L Na_2SO_4, 2 g/L KNO_3, 0.01 g/L $FeSO_4 \cdot 7H_2O$, and 1 mL/L trace metal solution. Anthracene at a concentration of 500 mg/L was added to the medium before it was autoclaved for 20 minutes at 121°C. After autoclaving, sterile $MgSO_4 \cdot 7H_2O$ was added to the medium to a concentration of 2 g/L.

3.1.3 Surfactants

Two surfactants were chosen for this investigation. Both a nonionic surfactant and an anionic surfactant were chosen in order to determine whether the type of surfactant had any effect on microbial adhesion. The nonionic surfactant was Triton X-100, which was obtained from Rohm and Haas Company of Canada Limited, West Hill, Ontario. Its head group is made up of 9 to 10 polyoxyethylene units, while its tail group is a branched 8 carbon unit, as shown in Figure 3.1. The anionic surfactant was Dowfax 8390, which was obtained from Dow Chemical Company, Midland, Michigan. Its head group is a diphenyl oxide disulfonate unit, while its tail group is a linear 16 carbon chain, as shown in Figure 3.2.

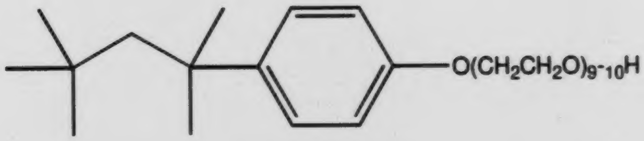

Figure 3.1: Chemical Structure of Triton X-100

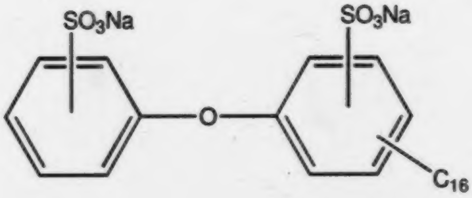

Figure 3.2: Chemical Structure of Dowfax 8390

3.1.4 Other Chemicals

Anthracene was obtained from Sigma Chemical Company, St. Louis, Missouri, and was reported to be 99% pure. Anhydrous D-glucose was obtained from BDH Inc., Toronto, Ontario. Methylene chloride was obtained from EM Science, Gibbstown, New Jersey, and was reported to be better than 99% pure.

3.2 METHODS

3.2.1 Determination of the Critical Micelle Concentration

The first step in this investigation was the determination of the critical micelle concentration (CMC) of each surfactant. This was done by preparing a number of solutions, each containing a different surfactant concentration in 50 mM potassium phosphate buffer at a pH of 7.2. The Triton X-100 concentrations ranged from 0.12 mM to 0.30 mM, while the Dowfax 8390 concentrations ranged from 0.2 mM to 1.1 mM. The surface tension of each solution was determined with a tensiometer, model #70545, manufactured by Central Scientific Company, Chicago, Illinois. The surface tension was plotted against surfactant concentration, and the point at which the surface tension no longer continued to decrease as the surfactant concentration increased was determined to be the CMC (Myers 1988). The CMC of Triton X-100 was determined to be 0.24 mM, which has also been reported elsewhere (Laha and Luthy 1992). The CMC of Dowfax 8390 was determined to be 0.8 mM, which is an order of magnitude lower than what has been reported elsewhere (Rouse *et al.* 1993). This will be discussed further in the next chapter.

3.2.2 Preparation of Organic Tars

The model hydrocarbon surfaces used in some parts of this investigation were organic tars. These tars were obtained by extracting the organic material from approximately 15 g of each soil with 200 mL of methylene chloride using a Soxhlet extraction apparatus over a period of 8 to 10 hours. The recovered organic material was transferred to glass jars that were left open overnight so that the methylene chloride could evaporate. The jars were then covered and stored at 4°C.

3.2.3 Preparation of Microbial Cultures

To prepare the microbial cultures for use, a vial of each microbial strain suspended in glycerol stock was removed from the freezer. The *Rhodococcus* species was streaked on plates of plate count agar (PCA) obtained from Difco Laboratories, and the *Pseudomonas* species was streaked on plates of trypticase soy agar (TSA) obtained from Difco Laboratories. The *Rhodococcus* species was incubated for 7 days, while the *Pseudomonas* species was incubated for 3 days. The incubation temperature was 27°C. At the end of the incubation period, the plates were stored at 4°C.

The microorganisms were transferred from their respective agar plates into 500 mL Erlenmeyer flasks, each of which contained 100 mL of the growth medium. The flasks also contained a 1.2 cm diameter steel coil to prevent the cells from aggregating. The flasks were then placed on a shaker in a room at 27°C, and were shaken at 200 rpm on a New Brunswick gyrotory shaker. Both microorganisms required 14 days to grow on anthracene before they could be used in experiments. It should be noted that the *Rhodococcus* species was able to utilize anthracene as its sole carbon source quite efficiently, while the *Pseudomonas* species did grow on anthracene even though it was clear that it would grow much better on other carbon sources.

The *Rhodococcus* species was checked for purity by being streaked on PCA. A sample was taken from each flask 3 or 4 days after being transferred into the growth medium, and the streaked plates were incubated at 27°C for 7 days. The *Pseudomonas* species was checked for purity by being streaked on TSA. A sample was taken from each flask 6 or 7 days after being transferred into the growth medium, and the streaked plates were incubated at 27°C for 3 days. Plates that showed that the cultures were pure were stored at 4°C, and were used to inoculate the next batch of growth medium.

3.2.4 Initial Adhesion Experiments

To determine whether or not surfactants affect the adhesion of microorganisms to the organic tars, the tars were dissolved in methylene chloride so that the concentration was 1 mg tar/mL methylene chloride. Test tubes were filled with chromic acid and left overnight to ensure that the surface of each test tube was clean. The chromic acid was then rinsed thoroughly with distilled water and left to air dry. Then, 5 mL of each solution were transferred into a series of test tubes. The test tubes were placed in a roller test tube rack, which was rotated at 12 rpm so that the methylene chloride could evaporate overnight while the inside of the test tubes became coated with the tars.

A 50 mM solution of potassium phosphate buffer at a pH of 7.2 was prepared. The microbial cultures were centrifuged at approximately 16 200 x g for 10 minutes. The growth medium was decanted and the microorganisms were resuspended in the phosphate buffer. The microbial suspension was centrifuged again at approximately 16 200 x g for 10 minutes, the buffer was decanted, and the microorganisms were resuspended in buffer twice more.

Each microbial suspension was divided in three parts. One part was left without the addition of any surfactant, while surfactant was added to the other two parts so that one part contained 0.12 mM Triton X-100 and the other part contained 0.4 mM Dowfax 8390, or approximately half the respective CMCs. Solutions of buffer containing 0.12 mM Triton X-100 and 0.4 mM Dowfax 8390 were also prepared to be used as controls along with the 50 mM phosphate buffer. The initial optical densities of each of these solutions were determined using a spectrophotometer at a wavelength of 600 nm (OD_{600}). The 50 mM phosphate buffer solution was used as the reference for all optical density readings.

Next, 5 mL of each solution were placed in the tar coated test tubes. These solutions were also placed in clean test tubes as controls. This was done in triplicate. All of the test tubes were then placed in a roller test tube rack, where they were rotated at 12 rpm for a period of three hours. They were then vortexed for 30 seconds each at a speed just above the threshold value so that the liquid level within the test tubes did not exceed the height of the coat of tar. The test tubes were then allowed to settle for 10 minutes. The tips of Pasteur pipettes were placed halfway between the meniscus and the bottom of each test tube, and approximately 1 mL of each sample was drawn so that its final OD_{600} could be determined.

3.2.5 Continued Adhesion Experiments

To determine the effects of the addition of a surfactant solution to a system in which the microorganisms were previously allowed to adhere to the organic tars, the test tubes were prepared with organic tars as before. Similarly, the microorganisms were removed from their growth medium and resuspended in 50 mM phosphate buffer at a pH of 7.2.

The initial OD_{600} of each microbial suspension was recorded using 50 mM phosphate buffer as the reference. Then, 5 mL of each microbial suspension were placed in test tubes containing the organic tars and in control test tubes without any tar. The 50 mM phosphate buffer was also placed in both tar coated test tubes and in clean test tubes. The test tubes were placed in a roller test tube rack and rotated at 12 rpm for three hours to allow the microorganisms to adhere to the test tube surface. Surfactant was then added to test tubes with tar and without tar, so that the final surfactant concentrations were 0.12 mM Triton X-100 and 0.4 mM Dowfax 8390. Some test tubes were left without surfactant as controls. This was done in triplicate. The test tubes were vortexed for 30 seconds, and left to settle for 10 minutes before the final OD_{600} values were determined.

3.2.6 Microbial Growth Experiments

To determine whether the presence of surfactant had any effect on the ability of the microorganisms to adhere to a crystalline carbon source in order for them to multiply, Erlenmeyer flasks containing 50 mg anthracene, 90 mL sterile growth medium with surfactant at the appropriate concentration, and a 10 mL suspension of the given species previously suspended in growth medium were prepared. The Erlenmeyer flasks containing the anthracene were autoclaved separately from the growth medium. Most of the anthracene particles were approximately 100 μm in diameter. The concentration of surfactant in each flask was either 0.12 mM Triton X-100 or 0.4 mM Dowfax 8390. Other flasks did not contain any surfactant so that they could be used as controls. Each set of conditions was repeated in quintuplicate. Samples were taken from each flask at the beginning of the experiment (day 0), and after 1, 2, 3, 5, 7, 10, and 14 days. The OD_{600} of each sample was recorded as an indication of microbial growth. This experiment was repeated using the *Pseudomonas* species and 50 mg glucose/100 mL liquid to determine the effects of surfactant on microbial growth in the presence of a soluble carbon source.

3.2.7 Packed Column Experiments

The effects of surfactants on the ability of microorganisms to adhere to surfaces in a packed column were determined. Glass columns with an internal diameter of 12 mm were filled with borosilicate glass beads to a height of 40 cm. The effluent flow valve had an internal diameter of 2 mm. The glass beads were soaked and shaken in 750 mL of a 5% nitric acid solution overnight in 750 g batches, rinsed four times with distilled water, washed once with 1 M potassium phosphate buffer at a pH of 7.2, rinsed once more with distilled water, and left to air dry.

Each microorganism was removed from its growth medium and resuspended in 50 mM phosphate buffer, as described above. Each microbial suspension was divided into three parts. One part did not contain any surfactant, and surfactant was added to the other parts so that one part contained 0.12 mM Triton X-100 and one part contained 0.4 mM Dowfax 8390. The solutions were fed through the top of the columns, and the effluents were collected in 2 mL aliquots by a 7000 Ultrorac fraction collector, distributed by Fisher Scientific Limited. The cell suspensions were able to migrate through the column at a rate so that approximately 12 mL of the effluent could be collected per minute. The initial feed contained 60 mL microbial suspension followed by 20 mL of 50 mM phosphate buffer.

This was done in triplicate. This experiment was repeated using 40 mL microbial suspension followed by 20 mL of 50 mM phosphate buffer.

4.0 RESULTS AND DISCUSSION

The goal of this study was to determine whether or not the presence of surfactants has any effect on the adhesion of two species of microorganisms to hydrophobic surfaces. This study is the first step in developing a detailed mechanism that will explain surfactant-hydrocarbon-microorganism interactions. All raw data may be found in Appendix A, and sample calculations may be found in Appendix B. Statistical analyses may be found in Appendix C.

4.1 SURFACTANT CRITICAL MICELLE CONCENTRATIONS

The first step in this investigation was to determine the CMC of each surfactant in order to ensure that the concentrations of the surfactants would be below their CMCs.

4.1.1 Critical Micelle Concentration of Triton X-100

The surface tension of solutions of Triton X-100 was plotted against concentration of Triton X-100 suspended in 50 mM potassium phosphate buffer at a pH of 7.2. This was done using Triton X-100 that had previously been in contact with the tars, as well as Triton X-100 that had not been in contact with any tar, as shown in Figure 4.1. Each data point represents the average surface tension of two readings per sample, which may be found in Table A-1. The surface tension decreased linearly as the surfactant concentration increased from 0.12 mM to 0.24 mM. As the surfactant concentration increased from 0.24

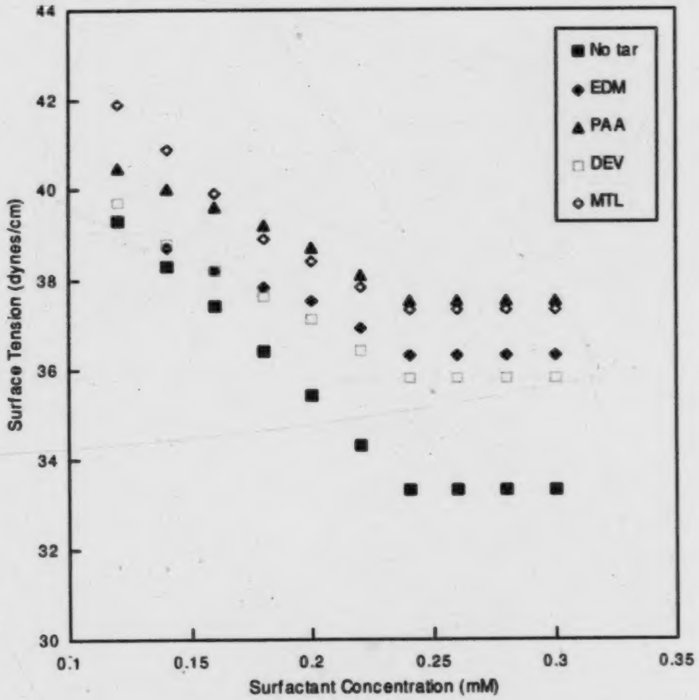

Figure 4.1: Determination of the CMC of Triton X-100 in the Presence and Absence of Organic Tars

mM to 0.30 mM, the surface tension remained constant. This trend occurred regardless of whether or not the surfactant solution had been in contact with any of the organic tars. Thus, the CMC of Triton X-100 was determined to be 0.24 mM in the presence and absence of the tars. The shift in surface tension in the presence of the tars may be accounted for by solubilization of some of the tar components. A concentration of 0.12 mM Triton X-100, or one half of its CMC, was selected in this investigation.

The CMC of Triton X-100 in the presence of the *Rhodococcus* species was also determined and compared to that in the absence of any microorganisms, as shown in Figure 4.2. The raw data may be found in Table A-2. This figure shows that the presence of this species suspended in potassium phosphate buffer to an OD_{600} of approximately 0.6 did not affect the CMC of this surfactant. Thus, neither the surfaces of the cells nor the surfaces of the tars were able to adsorb enough of the surfactant to alter the CMC. Within each test tube, the available surface area to volume of liquid ratio was determined to be approximately 9.4 cm^2/cm^3, as shown in section B.1. Thus, it is unlikely that there was monolayer coverage of surfactant monomers onto the tars. Low levels of adsorption, however, may have occurred. It is possible that the surface of the glass was able adsorb the same amount of surfactant as the tars. Further work would be required to determine whether surfactant monomers preferentially adsorb to cell surfaces or to tar surfaces.

It should be noted that the surface tensions of the blank solutions in Figure 4.1 were lower than the corresponding surface tensions of the same solutions in Figure 4.2. This discrepancy may be accounted for by a difference in laboratory temperatures on the days when the different samples were taken. It was found that an increase in temperature

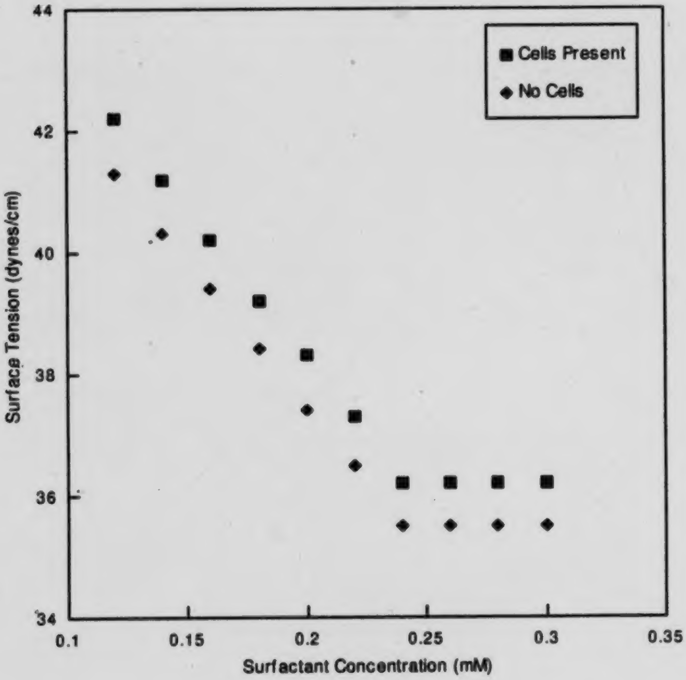

Figure 4.2: **Determination of the CMC of Triton X-100 in the Presence of the *Rhodococcus* Species**

of 0.2°C led to an increase in surface tension of approximately 0.4 dynes/cm within the range of 20°C to 23°C.

4.1.2 Critical Micelle Concentration of Dowfax 8390

The surface tension of Dowfax 8390 was plotted against concentration of Dowfax 8390 suspended in 50 mM potassium phosphate buffer. This was done using Dowfax 8390 that had previously been in contact with the tars, as well as with Dowfax 8390 that had not been in contact with any tar, as shown in Figure 4.3. The raw data may be found in Table A-3. The surface tension gradually decreased as the surfactant concentration increased from 0.2 mM to 0.8 mM. As the surfactant concentration increased from 0.8 mM to 1.1 mM, the surface tension remained constant. Thus, the CMC of Dowfax 8390 suspended in 50 mM potassium phosphate buffer at a pH of 7.2 was determined to be approximately 0.8 mM. The lack of a sharp CMC may have been the result of a heterogeneous surfactant mixture, as well as the relatively large increments of 0.1 mM used to cover a broad range of surfactant concentrations. The CMC remained the same even when the surfactant solutions had been in contact with the tars. As a result, it was decided that a concentration of 0.4 mM Dowfax 8390, or one half of its CMC, would be used in this investigation.

The CMC of Dowfax 8390 in the presence of the *Rhodococcus* species was also determined and compared to the CMC of the surfactant in the absence of any microorganisms, as shown in Figure 4.4, and the raw data may be found in Table A-4. It was found that the presence of this species suspended in potassium phosphate buffer at an OD_{600} of approximately 0.6 does not affect the CMC of the surfactant.

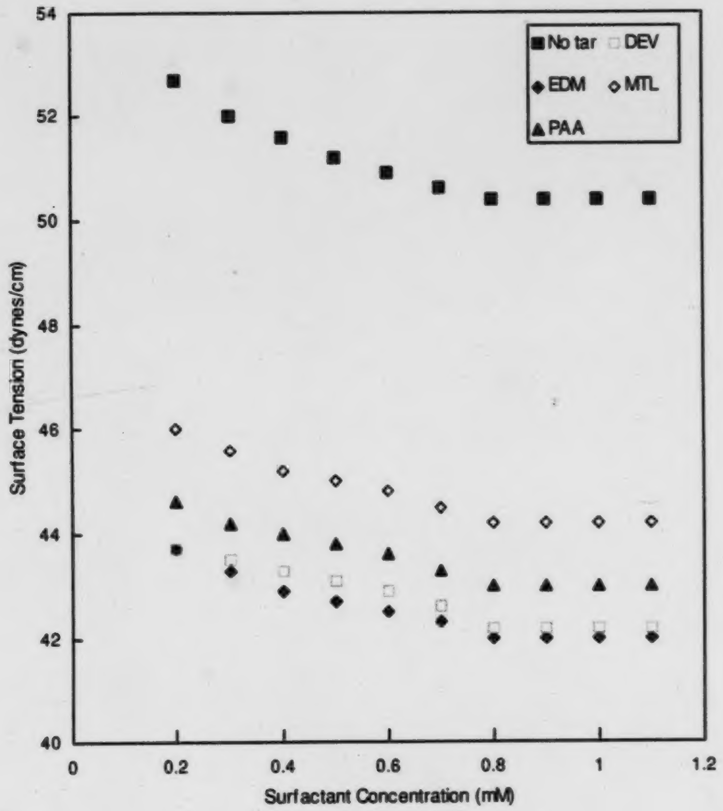

Figure 4.3: Determination of the CMC of Dowfax 8390 in the Presence and Absence of Organic Tars

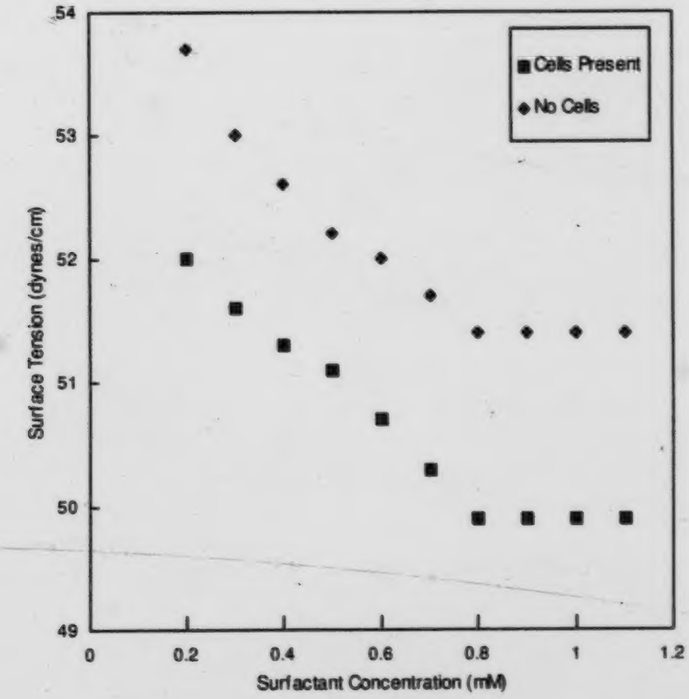

Figure 4.4: **Determination of the CMC of Dowfax 8390 in the Presence of the *Rhodococcus* Species**

4.2 INITIAL ADHESION OF MICROORGANISMS TO ORGANIC TARS

The adhesion capability of both microorganisms to each organic tar in the presence of surfactants was compared to the adhesion capability of both microorganisms to each organic tar in the absence of surfactants. This experimental design ensured that the effects of the two different types of surfactants on the initial adhesion of the microorganisms to an organic substrate could be determined. This experiment was done twice to ensure that the results could be reproduced.

4.2.1 Initial Adhesion of the *Rhodococcus* Species to Organic Tars

The percentage of *Rhodococcus* cells that adhered to both the tar coated test tubes and the clean test tubes in the presence and absence of surfactant is shown in Tables 4.1 and 4.2. The data reported in these tables represent the average OD_{600} of samples taken from three test tubes, with the exception of the final OD_{600} of samples taken from the test tubes that did not contain either surfactant. These data represent the average OD_{600} of samples taken from six test tubes. The data may be found in Tables A-5 and A-6, and the calculations may be found in section B.2. The final OD_{600} was taken once from each test tube. The results demonstrated that the *Rhodococcus* species adhered to surfaces better in the absence of surfactant than in the presence of surfactant. Approximately 40% of the cells adhered to the four tars in the absence of surfactant, while closer to 50% of the cells adhered to the surface of the clean glass test tubes in the absence of surfactant.

Table 4.1: Initial Adhesion of *Rhodococcus* Cells to Organic Tars (Series 1)

Tar	Solution	Initial O.D.	Cells Final O.D.	No cells Final O.D.	% cells adhered to surface
EDM	Buffer	0.587	0.358	0.009	41
	Triton	0.592	0.580	0.089	17
	Dowfax	0.587	0.442	0.065	36
PAA	Buffer	0.587	0.343	0.012	44
	Triton	0.592	1.369	0.815	6
	Dowfax	0.587	0.757	0.233	11
DEV	Buffer	0.587	0.358	0.012	41
	Triton	0.592	1.177	0.622	6
	Dowfax	0.587	0.419	0.032	34
MTL	Buffer	0.587	0.359	0.000	39
	Triton	0.592	0.571	0.007	5
	Dowfax	0.587	0.449	0.005	24
None	Buffer	0.587	0.301	0	49
	Triton	0.592	0.562	0	5
	Dowfax	0.587	0.473	0	19

Table 4.2: Initial Adhesion of *Rhodococcus* Cells to Organic Tars (Series 2)

Tar	Solution	Initial O.D.	Cells Final O.D.	No cells Final O.D.	% cells adhered to surface
EDM	Buffer	0.587	0.367	0.014	40
	Triton	0.600	0.586	0.098	19
	Dowfax	0.604	0.451	0.029	30
PAA	Buffer	0.587	0.379	0.011	37
	Triton	0.600	1.395	0.812	3
	Dowfax	0.604	0.830	0.259	5
DEV	Buffer	0.587	0.358	0.008	40
	Triton	0.600	1.151	0.590	7
	Dowfax	0.604	0.456	0.021	28
MTL	Buffer	0.587	0.377	0.000	36
	Triton	0.600	0.592	0.006	2
	Dowfax	0.604	0.498	0.002	18
None	Buffer	0.587	0.287	0	51
	Triton	0.600	0.596	0	1
	Dowfax	0.604	0.486	0	20

Conversely, fewer than 10% of the cells adhered to the PAA tar, DEV tar, MTL tar, and glass in the presence of Triton X-100, and fewer than 20% of the cells adhered to the EDM tar in the presence of this surfactant. Similarly, the percentage of cells that adhered to the various surfaces in the presence of Dowfax 8390 ranged from 18% to 36%. In the absence of microorganisms, the final OD_{600} of both surfactants suspended in buffer in the clean test tubes was determined to be 0. The *Rhodococcus* species was able to adhere to both the glass test tube surface and the organic tars. The presence of both surfactants inhibited adhesion, however the presence of Triton X-100 at a concentration of half of its CMC inhibited adhesion more effectively than the presence of Dowfax 8390 at a concentration of half of its CMC. Further work would be required to determine whether lower surfactant concentrations would inhibit adhesion to a lesser extent, and whether there is a surfactant concentration at which adhesion would not be inhibited.

4.2.2 Initial Adhesion of the *Pseudomonas* Species to Organic Tars

The percentage of *Pseudomonas* cells that adhered to both the tar coated test tubes and the clean test tubes in the presence and absence of surfactant is shown in Tables 4.3 and 4.4. All data in these tables represent the average OD_{600} of samples taken from three test tubes, with one sample being taken from each test tube. The data may be found in Tables A-7 and A-8. Cells of this species were also more likely to adhere to surfaces in the absence of surfactant than in the presence of either surfactant. Approximately 40 to 50% of the cells adhered to the organic tars in the absence of surfactant, while fewer than 30%

Table 4.3: Initial Adhesion of *Pseudomonas* Cells to Organic Tars (Series 1)

Tar	Solution	Initial O.D.	Cells Final O.D.	No cells Final O.D.	% cells adhered to surface
EDM	Buffer	0.576	0.275	0.002	53
	Triton	0.589	0.602	0.091	13
	Dowfax	0.589	0.577	0.025	6
PAA	Buffer	0.576	0.290	0.002	50
	Triton	0.589	1.232	0.809	28
	Dowfax	0.589	0.747	0.241	14
DEV	Buffer	0.576	0.341	0.010	43
	Triton	0.589	1.050	0.605	25
	Dowfax	0.589	0.521	0.023	15
MTL	Buffer	0.576	0.363	0.000	37
	Triton	0.589	0.488	0.004	18
	Dowfax	0.589	0.575	0.000	2
None	Buffer	0.576	0.352	0	39
	Triton	0.589	0.497	0	16
	Dowfax	0.589	0.527	0	11

Table 4.4: Initial Adhesion of *Pseudomonas* Cells to Organic Tars (Series 2)

Tar	Solution	Initial O.D.	Cells Final O.D.	No cells Final O.D.	% cells adhered to surface
EDM	Buffer	0.574	0.277	0.003	52
	Triton	0.607	0.604	0.096	16
	Dowfax	0.605	0.576	0.029	10
PAA	Buffer	0.574	0.296	0.002	49
	Triton	0.607	1.229	0.807	30
	Dowfax	0.605	0.743	0.250	18
DEV	Buffer	0.574	0.362	0.008	38
	Triton	0.607	1.071	0.606	23
	Dowfax	0.605	0.531	0.033	18
MTL	Buffer	0.574	0.365	0.000	36
	Triton	0.607	0.497	0.004	19
	Dowfax	0.605	0.571	0.000	6
None	Buffer	0.574	0.383	0	33
	Triton	0.607	0.519	0	14
	Dowfax	0.605	0.544	0	10

of the cells were able to adhere to any of the organic tars in the presence of Triton X-100 and fewer than 20% of the cells were able to adhere to any of the organic tars in the presence of Dowfax 8390. Similarly, approximately 35% of the cells were able to adhere to the clean glass in the absence of surfactant, while approximately one half of that amount adhered to the glass in the presence of Triton X-100, and approximately one third of that amount adhered to the glass in the presence of Dowfax 8390. Thus, the presence of both surfactants inhibited adhesion. However, the presence of Dowfax 8390 at a concentration of half of its CMC inhibited adhesion more effectively than the presence of Triton X-100 at a concentration of half of its CMC. Further work would be required to determine whether lower surfactant concentrations would inhibit adhesion to a lesser extent, and whether there is a surfactant concentration at which adhesion would not be inhibited.

4.2.3 Implications of Surfactant Use on Initial Adhesion

In order for biodegradation to occur, the microorganisms must be able to contact the target compounds. Such contact can occur either by dissolution of the target compounds into the aqueous phase, or by adhesion of the microorganisms directly onto the hydrocarbon surfaces. The latter mechanism has been stressed in the case of liquid hydrocarbons such as hexadecane (Rosenberg and Rosenberg 1981; Rosenberg and Rosenberg 1985), while dissolution has been emphasized in the degradation of solid polycyclic aromatic hydrocarbons (Aronstein and Alexander 1992; Liu *et al.* 1995; Volkering *et al.* 1995b). The results of the present study clearly show that microorganisms are able to adhere to viscous tars, although adhesion was inhibited in the presence of surfactants at concentrations well below CMC.

Similar inhibition of adhesion by surfactants has been reported for liquid hydrocarbon surfaces. The presence of Triton X-100 at a concentration above its CMC inhibited the adhesion of a Gram positive microorganism to a heptamethylnonane-water interface, which prevented the microorganism from degrading both hexadecane and naphthalene in the heptamethylnonane phase (Efroymson and Alexander 1991; Ortega-Calvo and Alexander 1994). The adhesion of both a Gram negative microorganism and a Gram positive microorganism to octane was also inhibited by the presence of emulsan (Rosenberg *et al.* 1983a). A correlation between the ability of a surfactant to decrease the surface tension and the ability of the surfactant to inhibit adhesion has been noted (Whitekettle 1991). In that study, however, it was found that microbial adhesion was prevented in the presence of nonionic surfactants, while microbial adhesion was not prevented in the presence of anionic surfactants. All of the surfactants in that study were used at a concentration of 10 μg/mL, regardless of the CMC of the surfactant (Whitekettle 1991). This may be significant since nonionic surfactants tend to have lower CMCs than anionic surfactants.

The purpose of this experiment was to determine the effect of the presence of low concentrations of surfactants on the adhesion of microorganisms to a solid substrate, as opposed to liquid hydrocarbons. The results show that the conclusion reached in other studies that the presence of surfactants inhibits the adhesion of microorganisms to liquid hydrocarbons may be extended to solid and semi-solid substrates.

Hydrophobic interactions regulate the adhesion of microorganisms to hydrocarbons (Rosenberg *et al.* 1981). Because of their amphipathic structure, surfactant molecules are able to coat such surfaces with a thin layer so that the hydrophilic heads are in the aqueous phase while the hydrophobic tails are adsorbed onto the surface of the hydrocarbons. Thus, it appears that the ability of a surfactant to inhibit microbial adhesion to hydrophobic surfaces is the result of the formation of this thin surfactant film, which makes hydrophobic surfaces become more hydrophilic. Because the microorganisms that are more likely to adhere to the hydrophobic materials tend to be hydrophobic themselves, the increased hydrophilicity of the surface decreases the tendency of the microorganisms to adhere to such surfaces.

The MTL soil had been partially bioremediated before the commencement of the study, and its tarry extract was able to remain adhered to the surface of the test tube, even in the presence of both surfactants. Conversely, the presence of Triton X-100 led to the removal of a significant amount of the other tars, and the presence of Dowfax 8390 led to the removal of some of the tar. In the absence of surfactant, virtually none of the tar was removed from the test tubes. The test tubes were sampled at a depth midway between the meniscus and the bottom of the test tube in order to minimize the removal of the mobilized tars. It was assumed that the same amount of tar was removed from a given test tube in the presence of microorganisms as in the absence of microorganisms, so the OD_{600} corresponding to a given tar in the absence of microorganisms was subtracted from the OD_{600} corresponding to that tar in the presence of microorganisms. It was found that for each microorganism, the OD_{600} increased linearly with microbial concentration to an

OD_{600} of approximately 0.6. For the *Rhodococcus* species, an OD_{600} of 0.1 corresponded to a cell count of approximately 10^8 cfu/mL (Tongpim and Pickard 1996). The relationship between OD_{600} and cell count was not determined for the *Pseudomonas* species.

A t-test was performed in order to determine whether there was a statistically significant difference between the affinity of the microorganisms for the tars and for the glass in the absence of surfactants. The method used is detailed in section B.3. The mean values representing the percentage of *Rhodococcus* cells that adhered to the glass and the percentage of *Rhodococcus* cells that adhered to each tar were compared, and may be found in Tables C-1 and C-2. By using a pooled estimate of the standard deviation, it was determined that there would be no difference between the affinity of the *Rhodococcus* cells for the glass and for each tar if the difference between the mean values was approximately 3% or less, to a confidence of 95%. Since this difference was greater than 3% for each tar, it may be concluded that the *Rhodococcus* cells have a statistically significant higher affinity for the glass than for the tars. Conversely, it may only be concluded that the affinity of the *Pseudomonas* cells for the tars was the same as for the glass if the difference between the means was approximately 5% or less, to a confidence of 95%, as shown in Tables C-3 and C-4. Thus, there was no statistically significant difference between the affinity of these cells for either the DEV tar or the MTL tar and the affinity of these cells for the glass, however the cells did have a statistically significant higher affinity for the other tars than for the glass.

Another interesting observation that should be noted is that Dowfax 8390 was more effective at suppressing the adhesion of the *Pseudomonas* species than the adhesion of the *Rhodococcus* species, while the reverse was true for Triton X-100. This may be explained by the cell wall structure of each species. The cell walls of many Gram positive microorganisms, such as *Rhodococcus* species, are more hydrophobic than those of Gram negative microorganisms (Stephens and Dalton 1987). This would indicate that the *Rhodococcus* species would be more likely to interact with a surfactant that does not carry any charge. Conversely, the cell wall of the *Pseudomonas* species would carry more of a charge, so it would be more likely to interact with a charged surfactant. This hypothesis may be verified by repeating the experiment using a variety of Gram positive and Gram negative microorganisms.

4.3 REMOVAL OF MICROORGANISMS FROM ORGANIC TARS

The microorganisms were allowed to adhere to organic tars before any surfactant was added to the test tubes. This was done in order to determine whether the microorganisms could remain adhered to a given surface after surfactants were added to a system. This experiment was done twice to ensure that the results could be reproduced.

4.3.1 Removal of the *Rhodococcus* Species from Organic Tars

The percentage of *Rhodococcus* cells that remained adhered to both the tar coated test tubes and the clean test tubes after surfactant was added was compared to the percentage of *Rhodococcus* cells that remained adhered to the test tubes to which no surfactant was added. The results are shown in Tables 4.5 and 4.6. All data in these tables represent the average OD_{600} of samples taken from three test tubes, with one sample being taken from each test tube. The raw data may be found in Tables A-9 and A-10. While approximately 30 to 50% of the cells remained adhered to each surface if no surfactant was added to the test tubes, fewer cells remained adhered to the surface if one of the surfactants was added to the test tubes. Fewer than 20% of the cells remained adhered to the clean glass as well as the EDM, PAA, and MTL tars in the presence of Triton X-100, while approximately 30% of the cells remained adhered to the DEV tar. Similarly, fewer than 30% of the cells remained adhered to all surfaces after Dowfax 8390 was added to the test tubes. Thus, the addition of either surfactant at a concentration of half of its CMC caused the removal of *Rhodococcus* cells from surfaces.

4.3.2 Removal of the *Pseudomonas* Species from Organic Tars

The number of *Pseudomonas* cells that remained adhered to both the tar coated test tubes and the clean test tubes after surfactant was added was compared to the number of cells that remained adhered to the test tubes to which no surfactant was added. The results are shown in Tables 4.7 and 4.8. All data in these tables represent the average OD_{600} of samples taken from three test tubes, with one sample being taken from each test tube. The raw data may be found in Tables A-11 and A-12. Approximately 35 to 50% of the cells remained adhered to each surface if no surfactant was added to the test tubes. Fewer cells remained adhered to the clean test tubes, the EDM tar, and the PAA tar after either surfactant was added, and fewer cells remained adhered to the DEV tar after Triton X-100

Table 4.5: Removal of *Rhodococcus* Cells from Organic Tars (Series 1)

Tar	Solution Added	Initial O.D.	Cells Final O.D.	No Cells Final O.D.	% cells adhered to surface
EDM	None	0.608	0.418	0.001	31
	Triton	0.608	0.827	0.315	16
	Dowfax	0.608	0.455	0.006	26
PAA	None	0.608	0.296	0.000	51
	Triton	0.608	1.051	0.506	10
	Dowfax	0.608	0.629	0.042	3
DEV	None	0.608	0.304	0.003	50
	Triton	0.608	0.698	0.275	30
	Dowfax	0.608	0.577	0.083	19
MTL	None	0.608	0.401	0.000	34
	Triton	0.608	0.581	0.000	4
	Dowfax	0.608	0.560	0.000	8
None	None	0.608	0.291	0	52
	Triton	0.608	0.570	0	6
	Dowfax	0.608	0.589	0	3

Table 4.6: Removal of *Rhodococcus* Cells from Organic Tars (Series 2)

Tar	Solution Added	Initial O.D.	Cells Final O.D.	No Cells Final O.D.	% cells adhered to surface
EDM	None	0.602	0.421	0.001	30
	Triton	0.602	0.833	0.317	14
	Dowfax	0.602	0.452	0.007	26
PAA	None	0.602	0.300	0.001	50
	Triton	0.602	1.065	0.493	5
	Dowfax	0.602	0.627	0.048	4
DEV	None	0.602	0.301	0.004	51
	Triton	0.602	0.694	0.278	31
	Dowfax	0.602	0.578	0.080	17
MTL	None	0.602	0.398	0.000	34
	Triton	0.602	0.582	0.000	3
	Dowfax	0.602	0.561	0.000	7
None	None	0.602	0.291	0	52
	Triton	0.602	0.567	0	6
	Dowfax	0.602	0.587	0	3

Table 4.7: Removal of *Pseudomonas* Cells from Organic Tars (Series 1)

Tar	Solution Added	Initial O.D.	Cells Final O.D.	No Cells Final O.D.	% cells adhered to surface
EDM	None	0.596	0.288	0.001	52
	Triton	0.596	0.776	0.335	26
	Dowfax	0.596	0.514	0.003	14
PAA	None	0.596	0.299	0.001	50
	Triton	0.596	0.900	0.513	35
	Dowfax	0.596	0.445	0.037	31
DEV	None	0.596	0.339	0.001	43
	Triton	0.596	0.788	0.280	15
	Dowfax	0.596	0.407	0.085	46
MTL	None	0.596	0.374	0.000	37
	Triton	0.596	0.383	0.000	36
	Dowfax	0.596	0.384	0.001	36
None	None	0.596	0.394	0	34
	Triton	0.596	0.503	0	16
	Dowfax	0.596	0.475	0	20

Table 4.8: Removal of *Pseudomonas* Cells from Organic Tars (Series 2)

Tar	Solution Added	Initial O.D.	Cells Final O.D.	No Cells Final O.D.	% cells adhered to surface
EDM	None	0.603	0.296	0.000	51
	Triton	0.603	0.783	0.322	24
	Dowfax	0.603	0.531	0.003	13
PAA	None	0.603	0.311	0.001	49
	Triton	0.603	0.908	0.499	32
	Dowfax	0.603	0.464	0.031	28
DEV	None	0.603	0.341	0.001	44
	Triton	0.603	0.803	0.283	14
	Dowfax	0.603	0.423	0.090	45
MTL	None	0.603	0.384	0.000	36
	Triton	0.603	0.390	0.001	35
	Dowfax	0.603	0.386	0.000	36
None	None	0.603	0.405	0	33
	Triton	0.603	0.515	0	15
	Dowfax	0.603	0.484	0	20

was added. Conversely, almost no cells were removed from the MTL tar after either surfactant was added, nor were any cells removed from the DEV tar after Dowfax 8390 was added. Thus, the addition of either surfactant at a concentration of half of its CMC caused the removal of *Pseudomonas* cells from surfaces in most cases. It appears that each surface may have its own properties that determine the surfactant concentration necessary for no removal to occur. In some instances, this concentration would be greater than half the CMC, but in many cases, this concentration would be less than half the CMC. Further studies would be required in order to relate surface properties of the substrate with surface properties of the cells, and how this relationship is affected by the presence of surfactants.

4.3.3 Implications of Surfactant Use on Previously Adhered Microorganisms

The above results show that both surfactants were able to remove the *Rhodococcus* species from both the surface of the glass test tubes and the surface of the organic tars. The surfactants were also able to remove the *Pseudomonas* species from most of these surfaces.

Similar phenomena have been observed by other research groups working with liquid hydrocarbons. For example, it has been noted that a high concentration of Triton X-100 was able to cause a Gram positive microorganism to be removed from a heptamethylnonane-water interface (Efroymson and Alexander 1991). Further, the presence of emulsan was able to cause both a Gram positive microorganism and a Gram negative microorganism to be removed from octane (Rosenberg *et al.* 1983a). The present study is the first that shows that the presence of either a nonionic surfactant or an anionic surfactant will cause microorganisms to be removed from organic tars.

This observation strengthens the hypothesis that hydrophobic interactions govern adhesion of microorganisms to hydrophobic surfaces. Because the surfactant monomers have a high affinity for such a surface, their hydrophobic tails would adhere to the surface once they are added to a system. The presence of their hydrophilic heads would, in turn, decrease the hydrophobicity of the surface, which would cause the hydrophobic microorganisms to have less of an affinity for the surface. Thus, the hydrophobic microorganisms would be released from the surface of the substrate.

Since the outer surface of the *Rhodococcus* species is regarded to be more hydrophobic than the outer surface of the *Pseudomonas* species, the presence of surfactants is more likely to cause the former to be released from the hydrophobic surface. This is probably why the presence of surfactant had little effect on the removal of the *Pseudomonas* species from some substrates.

As before, the MTL tar remained adhered to the surface of the glass test tubes, even after the addition of either surfactant. The presence of Triton X-100, however, caused some of the other tars to be removed from the surface of the test tubes. Less tar was removed from the surface of these test tubes as when the surfactant was added at the beginning of the experiment. The presence of Dowfax 8390 also caused some tar to be removed from the surface of the test tubes containing the EDM, PAA, and DEV tars.

A t-test was performed in order to determine whether there was a statistically significant difference between the affinity of the microorganisms for the tars and for the glass in the absence of surfactants. The mean values representing the percentage of *Rhodococcus* cells that adhered to the glass and the percentage of *Rhodococcus* cells that adhered to each tar were compared, as shown in Tables C-5 and C-6. In one series, it was determined that there would be no difference between the affinity of the *Rhodococcus* cells for the glass and for each tar if the difference between the mean values was approximately 6% to 10% or less to a confidence of 95%, and approximately 3% or less in the other series, to a confidence of 95%. The wide range in values may be due to the limited number of data points within each series. Nonetheless, the t-test suggests that there was no statistically significant difference between the affinity of the *Rhodococcus* cells for the both the PAA and DEV tars and the affinity of these cells for the glass, but the cells did have a statistically significant higher affinity for the glass than for the other tars. This is quite different than what was suggested from the results obtained in the initial adhesion experiment, and may be the result of slight changes in cell surface chemistry. Conversely, it may be concluded that the affinity of the *Pseudomonas* cells for the tars was the same as for the glass if the difference between the means was approximately 5% or less, to a confidence of 95%, as shown in Tables C-7 and C-8. Thus, there was no statistically significant difference between the affinity of these cells for the MTL tar and the affinity of these cells for the glass, but the cells did have a statistically significant higher affinity for the other tars than for the glass. This is the same result that was obtained in the initial adhesion experiment.

4.4 GROWTH OF MICROORGANISMS

The growth rates of both microorganisms in the presence of surfactants were compared to the growth rates of both microorganisms in the absence of surfactants. This was done to confirm the importance of adhesion to hydrocarbon surfaces in the microbial growth process.

4.4.1 Growth of the *Rhodococcus* Species

The OD_{600} of the *Rhodococcus* species growing on anthracene as a function of time in the presence and absence of surfactants are shown in Figure 4.5. The data may be found in Table A-13. In the absence of surfactant, the initial OD_{600} of the microbial suspension was approximately 0.12. The exponential phase lasted until approximately day 7, at which point the OD_{600} was slightly less than 1.2. At this time, the culture entered the stationary phase. In the presence of Triton X-100, the cell concentration increased more slowly from an OD_{600} of 0.17 to an OD_{600} of approximately 0.67 in the first seven days. Growth continued, giving an OD_{600} of approximately 0.81 after day 10, and a maximum sometime between day 10 and day 14. In the presence of Dowfax 8390, the OD_{600} reached a maximum of approximately 0.3 after five days, which was maintained for the duration of the experiment. In the absence of either surfactant, the liquid medium turned yellow as growth proceeded. This yellow color did not appear in the presence of either surfactant. The presence of surfactant may have caused some of the anthracene particles to disperse, however, this would have provided more surface area for transient adhesion. The presence

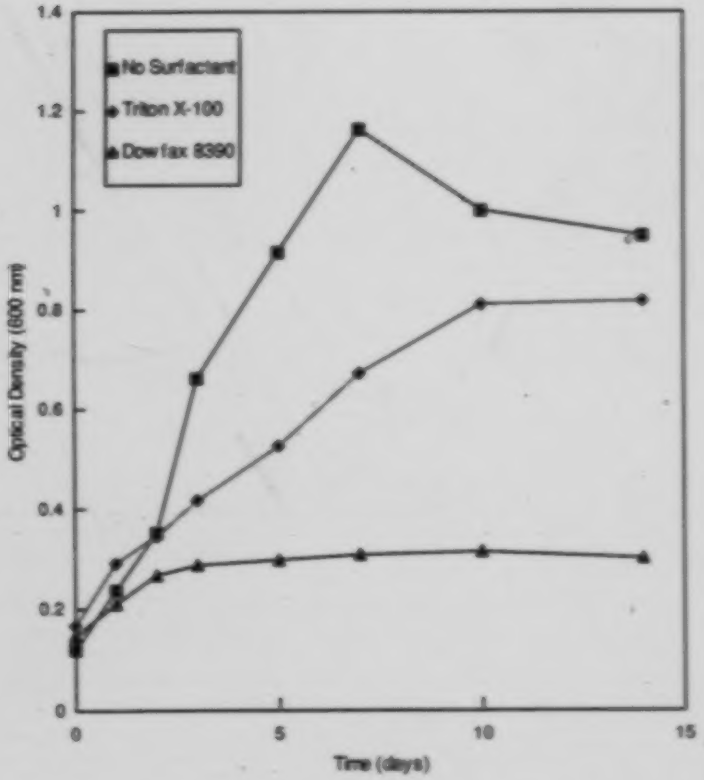

Figure 4.5: **Growth of the *Rhodococcus* Species on Anthracene in the Presence and Absence of Surfactants at a Concentration of Half of the CMC**

of either surfactant inhibited both the rate and the extent of growth, even at concentrations of half of the CMC.

4.4.2 Growth of the *Pseudomonas* Species

The OD_{600} of the *Pseudomonas* species growing on anthracene as a function of time in the presence and absence of surfactants are shown in Figure 4.6. The data may be found in Table A-14. In the absence of surfactant, the initial OD_{600} of the microbial suspension was approximately 0.06. The exponential phase lasted until approximately day 7, at which point the OD_{600} was approximately 0.34. Conversely, in the presence of Triton X-100, the OD_{600} increased to approximately 0.14 after 7 days, then decreased, while in the presence of Dowfax 8390, the OD_{600} increased to approximately 0.09 after 7 days before decreasing. In the absence of either surfactant, the liquid medium turned yellow as growth progressed. This yellow color did not appear in the presence of either surfactant. Thus, the presence of either surfactant inhibited both the rate and the extent of growth, even at concentrations of half of the CMC.

In the flasks that initially did not contain any surfactant, a layer of foam started to appear within about 2 days after inoculation. After about 5 days, the amount of bubbles and foaming within these flasks was similar to that in the flasks that initially contained Dowfax 8390. Thus, it was concluded that this species was able to produce its own biosurfactant, although no surface tension data was obtained.

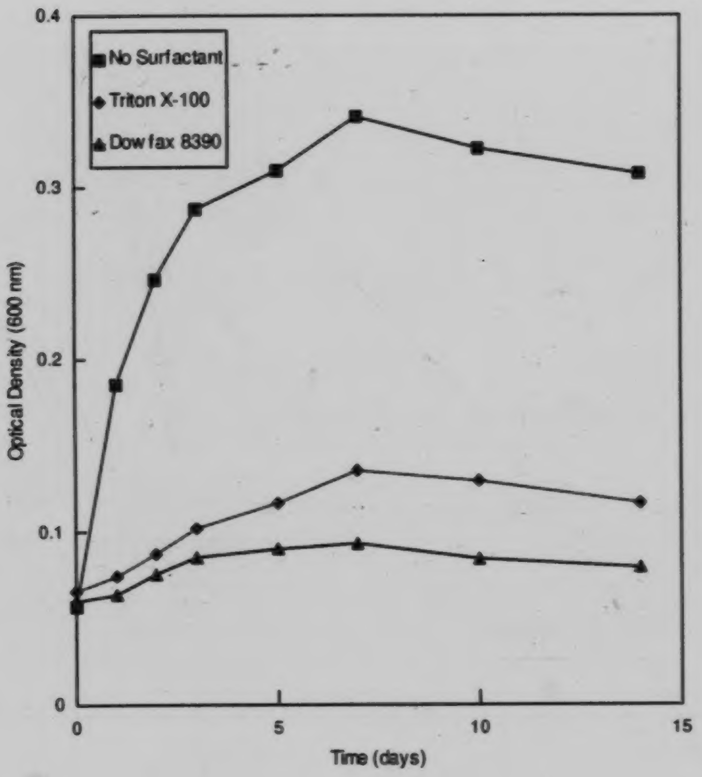

Figure 4.6: **Growth of the *Pseudomonas* Species on Anthracene in the Presence and Absence of Surfactants at a Concentration of Half of the CMC**

4.4.3 Implications of Surfactant Use on Microbial Growth

Clearly, the presence of either surfactant had a deleterious impact on the growth of both the *Rhodococcus* species and the *Pseudomonas* species. This observation indicates that either the surfactant molecules prevented the microorganisms from contacting the anthracene or the surfactants were toxic to the microorganisms.

Similar results have been reported elsewhere for liquids. For example, it was found that the presence of a nonionic surfactant caused yeast cells to detach from the *n*-alkane-water interface, leading to a decline in the growth rate of the culture (Aiba *et al.* 1969). The presence of nonionic surfactants had little effect on the growth rate of yeast cells on a soluble carbon source, although Triton X-100 did inhibit the onset of the exponential growth phase (Laouar *et al.* 1996). Further, the growth rate of a mixed culture on PCBs was inhibited in the presence of four nonionic surfactants, although it was noted that Triton X-100 was the least inhibitory (Viney and Bewley 1990). The presence of nonionic surfactants also inhibited the growth of a Gram positive microorganism on PAHs, although biodegradation of solubilized PAH was observed (Tiehm 1994). The explanation given was that the surfactants were toxic to the microorganisms, although if biodegradation of solubilized material could be observed, surfactant toxicity could not have been a factor. That study did not consider the possible inhibition of adhesion in the presence of surfactants. In the same study, it was noted that an anionic surfactant was used as a carbon source (Tiehm 1994).

The results of this experiment can be explained by two possible mechanisms. First, the surfactants may be toxic to the microorganisms and inhibit growth. Second, the results of this experiment may be explained by hydrophobic interactions. In the case of the latter, the hydrophobic tail of the surfactant would have a high affinity for the crystalline substrate, so the surfactant molecules would adsorb to the substrate. Once this occurred, the surfactant heads would create a hydrophilic film on the surface of the anthracene. Adhesion of the microorganisms to solid anthracene would tend to be transient in nature. This film would reduce the ability of the hydrophobic moieties of the microorganisms to adhere to the carbon source, thus preventing the microorganisms from obtaining enough carbon and reproducing. The previous results of this study showed that surfactants can inhibit adhesion to viscous and semi-solid tars. The results of this experiment extend these findings to suggest that this mechanism could also affect transient adhesion of the microorganisms to solid polycyclic aromatic hydrocarbons. Further studies would be required in order to determine whether there is a surfactant concentration at which no inhibition would occur. There was no evidence that either microorganism used in this study was able to use the surfactants as a carbon source.

4.4.4 Growth of the *Pseudomonas* Species in the Presence of Glucose

In order to eliminate the possibility that the surfactants were toxic to the microorganisms, the previous experiment was repeated using the *Pseudomonas* species and a soluble carbon source. Glucose was chosen to be the carbon source. In this experiment, the

Pseudomonas species was able to grow quite effectively within hours of inoculation, regardless of the presence or absence of surfactant, as shown in Figure 4.7. The data may be found in Table A-15. The initial OD_{600} was approximately 0.09, and increased to a maximum OD_{600} of approximately 1.0 within about eight hours. There was no significant difference between the three curves, indicating that the presence of either surfactant is not toxic to microorganisms. Thus, it may be concluded that if an insoluble carbon source is present, the presence of surfactants prevents the microorganisms from adhering to its surface by hydrophobic interactions.

4.5 MICROBIAL ADHESION IN A PACKED COLUMN

To determine whether the presence of surfactant had any effect on the adhesion or retardation of the microorganisms as they migrate, cell suspensions were fed through the top of a packed column, and the effluent was collected at the bottom. The percentage of cells suspended in a medium containing surfactant that were recovered was compared to the percentage of cells suspended in a medium without surfactant that were recovered. Glass was chosen as the model surface since previous experiments have shown that the microorganisms would preferentially adhere either to glass or to tars in the absence of surfactant. The void space within each column was determined to be approximately 17.3 mL, as shown in section B.4.

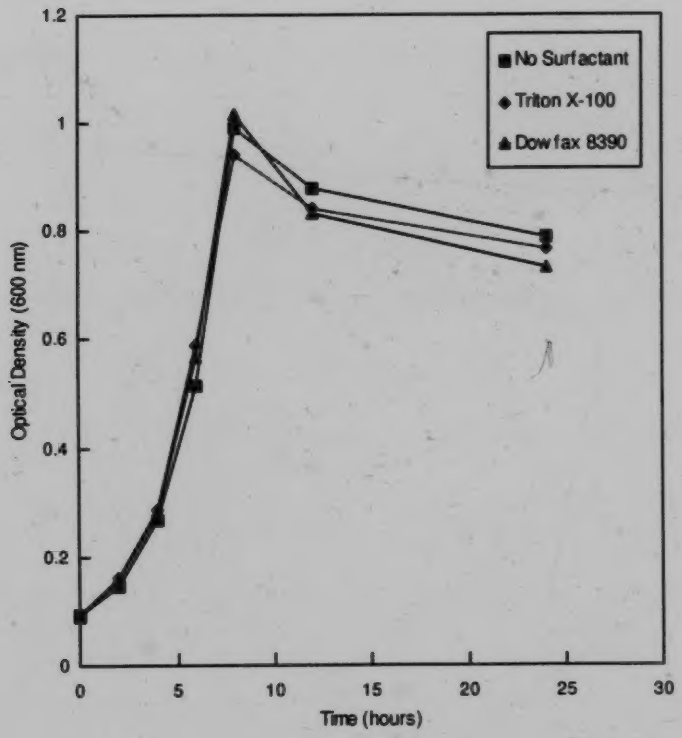

Figure 4.7: **Growth of the *Pseudomonas* Species on Glucose in the Presence and Absence of Surfactants at a Concentration of Half of the CMC**

4.5.1 Adhesion of the *Rhodococcus* Species in a Packed Column

The percentage of *Rhodococcus* cells that were recovered after 60 mL of a microbial suspension and 20 mL of potassium phosphate buffer were fed through the top of a column is shown in Figures 4.8, 4.9, and 4.10. The data may be found in Tables A-16, A-17, and A-18, and the calculation method may be found in section B.5. Approximately 33% of the cells suspended in a medium containing phosphate buffer only were recovered, while 40% and 58% of the cells suspended in media containing Triton X-100 and Dowfax 8390, respectively, could be recovered. Thus, approximately 67% of the cells that were not suspended in a medium that contained surfactant adhered to the surface of the glass, while approximately 60% and 42% of the cells that were suspended in media that contained Triton X-100 and Dowfax 8390, respectively, were able to adhere to the surface of the glass.

To verify that the amount of unrecovered liquid depended on the surface area within the column and the superficial velocity rather than microbial loading, the experiment was repeated using 40 mL of microbial suspension and 20 mL of potassium phosphate buffer. The percentage of cells recovered is shown in Figure 4.11, and the raw data may be found in Table A-19. Approximately 28% of the cells suspended in a medium containing phosphate buffer only were recovered, while 32% and 55% of the cells suspended in media containing Triton X-100 and Dowfax 8390, respectively, could be recovered. Thus, approximately 72% of the cells that were not suspended in a medium that contained surfactant adhered to the surface of the glass, while approximately 68% and

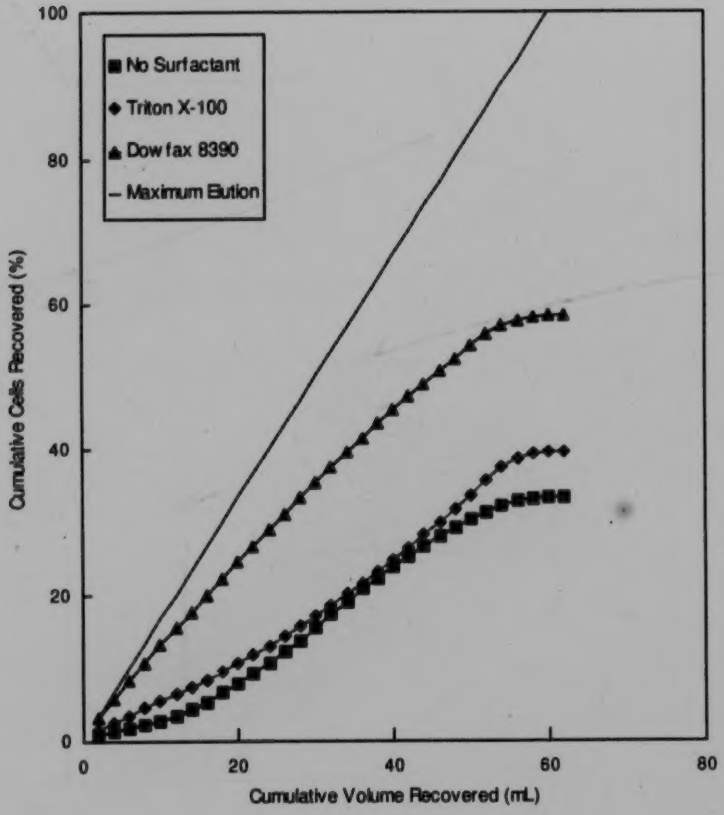

Figure 4.8: ***Rhodococcus*** **Cells Recovered from Packed Column (Series 1)**

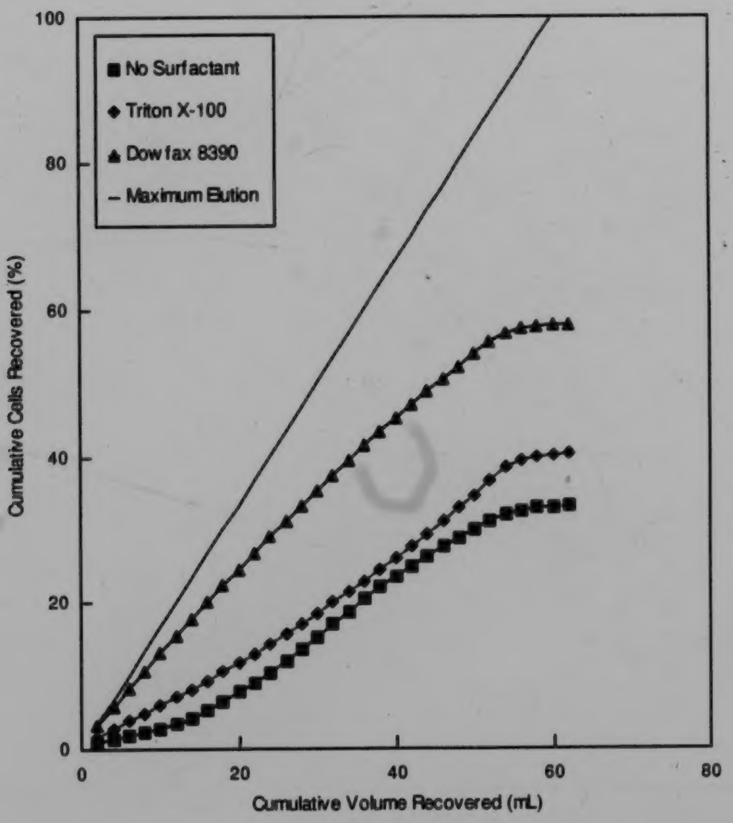

Figure 4.9: ***Rhodococcus*** **Cells Recovered from Packed Column (Series 2)**

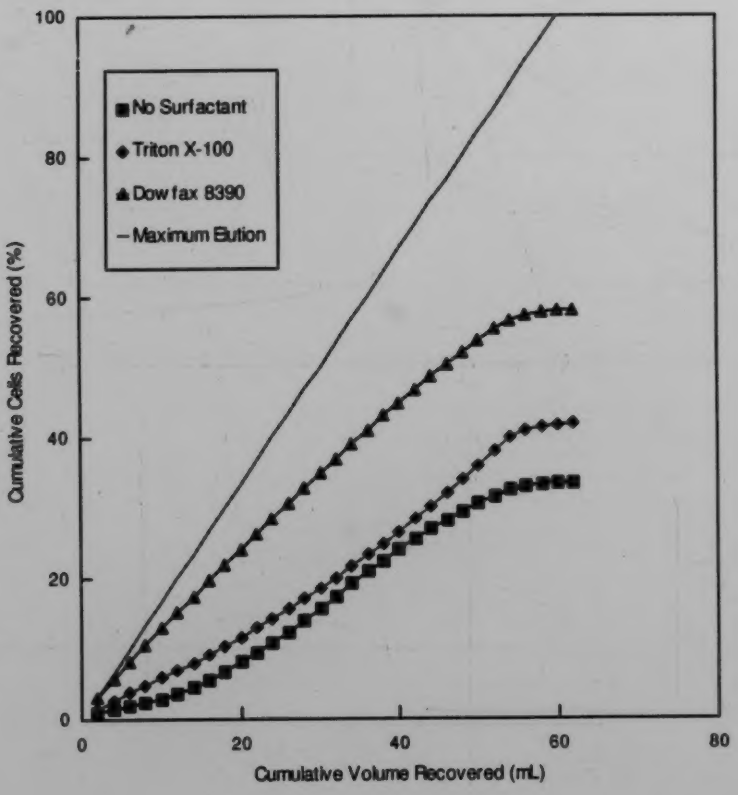

Figure 4.10: ***Rhodococcus*** **Cells Recovered from Packed Column (Series 3)**

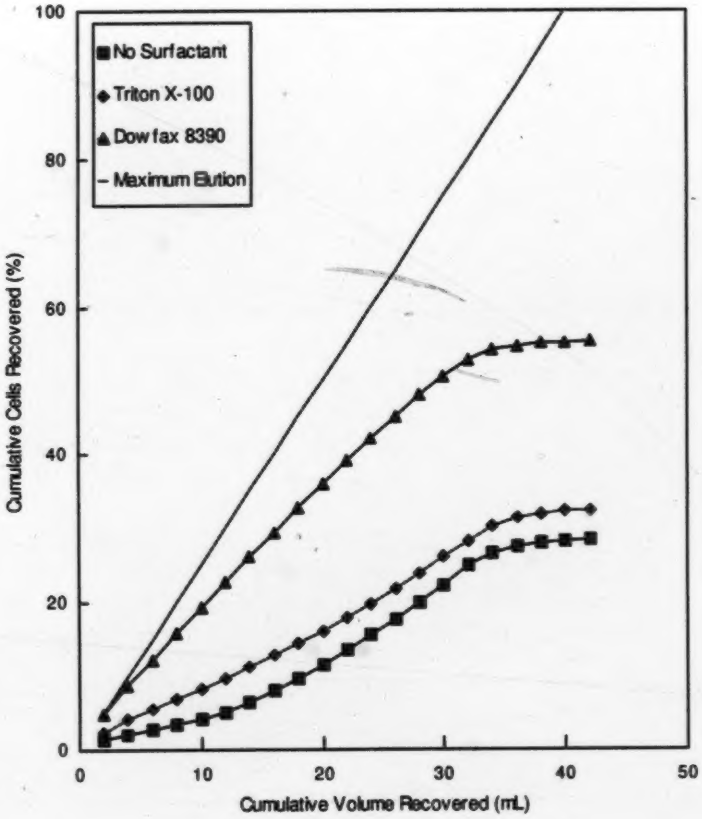

Figure 4.11: ***Rhodococcus*** **Cells Recovered from Packed Column With Reduced Loading**

45% of the cells that were suspended in media that contained Triton X-100 and Dowfax 8390 were able to adhere to the surface of the glass.

These results suggest that the number of cells that adhere to the surface of the glass beads and glass column is a function of available area and superficial velocity, and not microbial loading. The results also indicate that the presence of either a nonionic surfactant or an anionic surfactant reduces the adhesion of the *Rhodococcus* species to a glass surface, although the anionic surfactant has more of an inhibitory effect than the nonionic surfactant.

4.5.2 Adhesion of the *Pseudomonas* Species in a Packed Column

The percentage of *Pseudomonas* cells that were recovered after 60 mL of a microbial suspension and 20 mL of potassium phosphate buffer were fed through the top of a column is shown in Figures 4.12, 4.13, and 4.14. The raw data may be found in Tables A-20, A-21, and A-22. Regardless of the presence or absence of surfactant within the medium, approximately 85% of the cells could be recovered, while approximately 15% of the cells adhered to the glass substrate.

A t-test was performed in order to determine whether there was a statistically significant difference between the mean number of cells recovered in the absence of surfactant and the mean number of cells recovered in the presence of surfactant. By using a pooled estimate of the standard deviation, it was determined that there would be no difference in the adhesion of the microorganism in the presence or absence of surfactant if

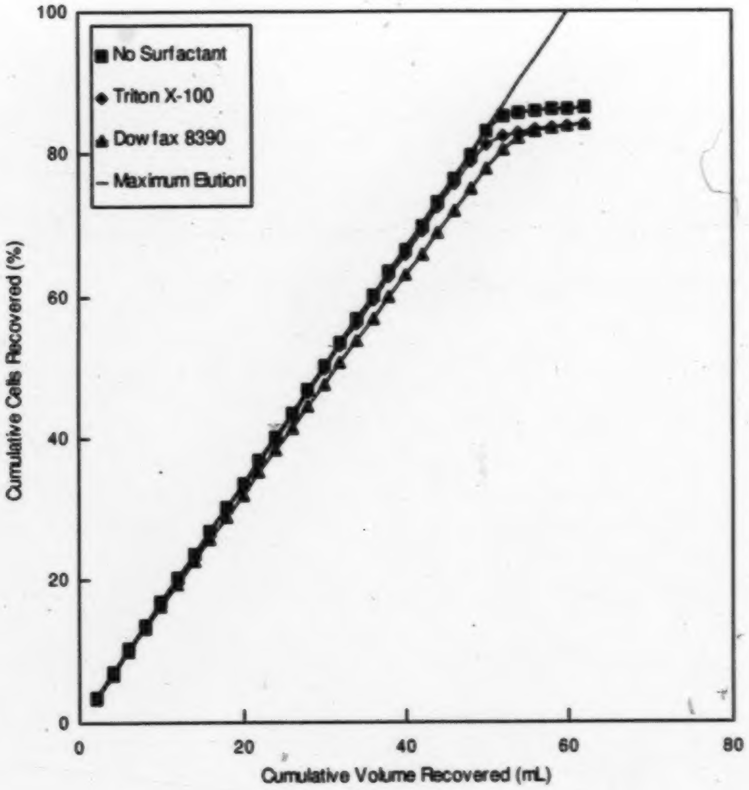

Figure 4.12: ***Pseudomonas*** **Cells Recovered from Packed Column (Series 1)**

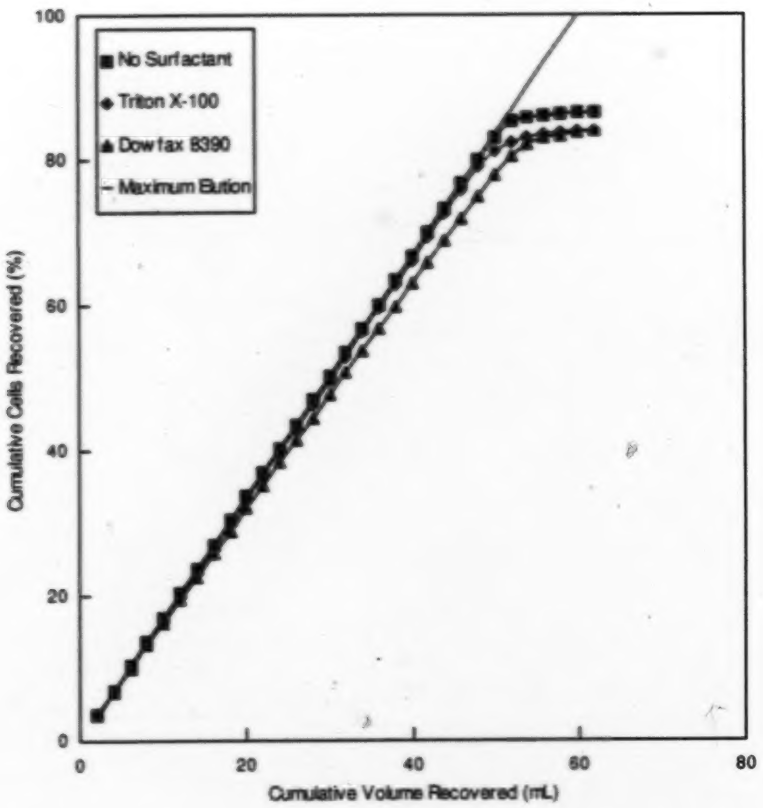

Figure 4.13: *Pseudomonas* Cells Recovered from Packed Column (Series 2)

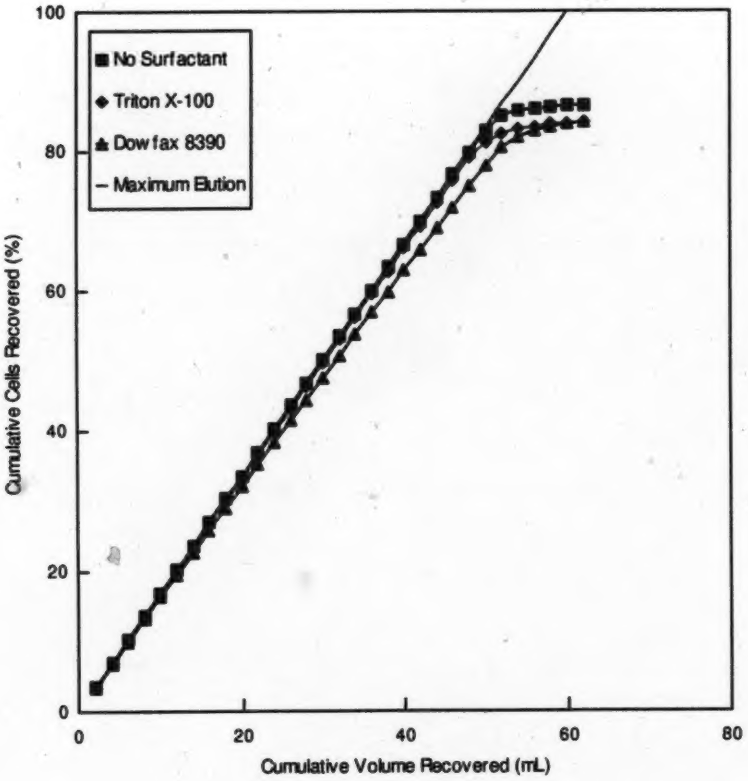

Figure 4.14: *Pseudomonas* Cells Recovered from Packed Column (Series 3)

the difference between the two means was less than 0.2% to a confidence of 95% for both surfactants, as shown in Table C-9. Thus, it would appear that the presence of surfactant slightly enhanced adhesion of the *Pseudomonas* cells to the glass substrate. It should be noted, however, that this difference is reflected only at the endpoint. Prior to flushing with buffer, the three curves are virtually identical, suggesting that the presence of surfactant had no effect on adhesion. Further, each mean represented the average of only three data points, and these three values were similar to one another. This meant that the standard deviation corresponding to each mean was small, and the resulting pooled estimate of the standard deviation was also small. These reproducible results were probably the result of using microorganisms from the same TSA plates to inoculate flasks at time intervals such that the microorganisms had been growing for a period of 14 days on the days the experiment was carried out.

This experiment was repeated using 40 mL of microbial suspension and 20 mL of potassium phosphate buffer. The percentage of cells recovered is shown in Figure 4.15, and the raw data may be found in Table A-23. As before, approximately 85% of the cells were recovered, while approximately 15% of the cells adhering to the glass substrate. There were slightly more cells that were not in contact with surfactant that could be recovered than cells that were in contact with both surfactants. As before, however, this difference was only reflected at the endpoint. Prior to flushing with buffer, elution curves were virtually identical under all three sets of conditions. Thus, it would appear that the presence of either surfactant at a concentration of half of its CMC had no effect on adhesion within the packed column.

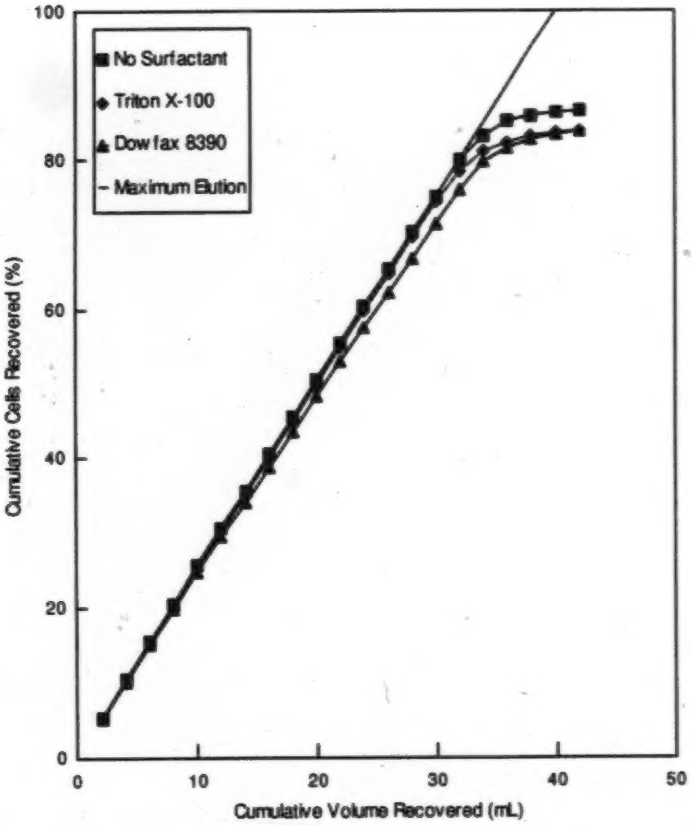

Figure 4.15: ***Pseudomonas*** **Cells Recovered from Packed Column With Reduced Loading**

4.5.3 Implications of Surfactant Use on Adhesion in a Packed Column

The number of *Rhodococcus* cells that adhered to the glass surface in the absence of surfactant exceeded the amount of *Rhodococcus* cells that adhered to the glass surface in the presence of either surfactant. This suggests that the surfactant molecules were able to sorb to the glass, creating a film between the aqueous phase and the glass. Within this film, the hydrophobic tails would be in contact with the glass surface while the hydrophilic heads would be in the aqueous phase. The layer of hydrophilic heads would then create an environment that would be unfavorable for the adhesion of hydrophobic microorganisms. A much smaller fraction of the *Pseudomonas* cells, whether they were in the presence of surfactant or not, were able to adhere to the hydrophobic surface. This may be because their outer cell surface is not nearly as hydrophobic as that of the *Rhodococcus* cells, so they are more likely to remain in the aqueous phase. As a result, the *Rhodococcus* cells would have a much greater affinity for surfaces than the *Pseudomonas* cells. Further experiments would be required to determine whether this trend is the same for other Gram negative and Gram positive microorganisms.

All three curves representing the elution of *Pseudomonas* cells in the presence and absence of surfactants coincided with the line that shows the maximum elution rate if no cell retention occurred until approximately 85% of the cells were eluted. Conversely, all three curves representing the elution of *Rhodococcus* cells in the presence and absence of surfactants were under the line that denotes the maximum elution rate. The curve representing the elution rate of *Rhodococcus* cells in the absence of surfactant was linear until approximately 30 mL of liquid was recovered, indicating that the percentage of cells that adhered within the column was a function of the volume of the loading until all adhesion sites were taken up. The curves representing the elution rate of *Rhodococcus* cells in the presence of either surfactant were S-shaped, indicating that there was some retention of cells initially, some of which would eventually be eluted as buffer was flushed through the column.

In the initial adhesion experiment, the cells were rotated in roller test tubes at a velocity of 12 rpm. Thus, within the test tubes, the cells had an effective velocity of approximately 60 cm/min before vortexing. Conversely, in the packed column experiment, the cells migrated through the column at a superficial velocity of approximately 11 cm/min, or an effective velocity of approximately 28 cm/min. The calculations may be found in section B.6. Thus, in the former experiment, the cells were exposed to a shear stress that was more than double that in the latter experiment. It has been found that microorganisms are more likely to adhere to surfaces under dynamic conditions than under static conditions if no surfactant is present (Rijnaarts *et al.* 1993). In this investigation, it was found that the *Rhodococcus* cells did adhere more effectively when they were exposed to a lower shear stress, but the opposite was true for the *Pseudomonas* cells. This would indicate that adhesion may be determined by the nature of the shear stress as well as the nature of the microbial cell wall. Further studies would be required in order to determine the relative significance of these effects.

5.0 CONCLUSIONS AND RECOMMENDATIONS

The critical micelle concentrations of Triton X-100 and Dowfax 8390 were determined to be 0.24 mM and 0.8 mM, respectively, in the presence and absence of both the tars and the *Rhodococcus* species. Thus, it was concluded that neither the surfaces of the cells nor the surfaces of the tars were able to adsorb enough of either surfactant to alter the CMC, although it is possible that the surface of the glass was able to adsorb the same amount of surfactant as the tars. Further studies may show whether surfactant monomers preferentially adsorb to cell surfaces or to tar surfaces.

Although other studies have shown that microorganisms are able to adhere to liquid hydrocarbons, the results of this study demonstrate that microorganisms are also able to adhere to semi-solid tars. The *Rhodococcus* species was able to adhere to the surface of the tars and the surface of the glass test tubes better in the absence of surfactant than in the presence of either surfactant. Although the presence of each surfactant at a concentration of half of its CMC inhibited the adhesion of this species, the presence of Triton X-100 had more of an inhibitory effect than the Dowfax 8390. The *Pseudomonas* species was also able to adhere to the surface of the tars and the surface of the glass test tubes better in the absence of surfactant than in the presence of either surfactant. In this case, however, the presence of Dowfax 8390 at a concentration of half of its CMC inhibited adhesion more effectively than the presence of Triton X-100 at a concentration of half of its CMC. Subsequent studies may determine whether lower surfactant concentrations would inhibit adhesion to a lesser extent, and whether there is a surfactant concentration at which adhesion would not be inhibited. The relationship between surfactant charge and cell wall hydrophobicity seems to be important in predicting the extent of inhibition of adhesion by the surfactants. This may be verified by testing the adhesion capabilities of various microorganisms in the presence of several surfactants.

Other studies have shown that microorganisms were removed from liquid hydrocarbons after the addition of surfactants, but the present study has shown that this result may be extended to semi-solid tars. The addition of either surfactant at a concentration of half of its CMC caused the removal of previously adhering *Rhodococcus* cells from the surface of both the glass and the tars. In most cases, the same was true for the *Pseudomonas* cells, although in other cases, no removal of cells occurred at this surfactant concentration. This would indicate that there is a surfactant concentration at which no cell removal would occur, and this concentration would depend on the relationship between the surface properties of the substrate and the surface properties of the cell. Further studies would be required in order to relate surface properties of the substrate with surface properties of the cell, and how this relationship is affected by the presence of surfactants.

This study was also able to show that the presence of surfactants will inhibit the adhesion of microorganisms to solid polycyclic aromatic hydrocarbons. The presence of both surfactants at concentrations of half of their respective CMCs will inhibit both the rate and the extent of growth of both the *Rhodococcus* species and the *Pseudomonas* species. A subsequent experiment in which glucose was used as the carbon source showed no inhibition of growth of the *Pseudomonas* species in the presence of either surfactant at a concentration of half of its CMC, indicating that neither surfactant was toxic to the microorganisms. Further studies may show that there is a threshold surfactant concentration below which inhibition of adhesion to solid polycyclic aromatic hydrocarbons will not occur.

The present study also showed that the presence of either surfactant at a concentration of half of its CMC will inhibit the adhesion of the *Rhodococcus* species to glass surfaces in a packed column, although Dowfax 8390 had more of an inhibitory effect than Triton X-100. Neither surfactant inhibited the adhesion of the *Pseudomonas* species, however very few of these cells were able to adhere to the glass in either the presence or absence of surfactant. This suggests that the nature of the shear stress also affects the affinity of the cells for surfaces. Further studies would be required to relate adhesion capability of different types of cells with the nature of the shear stress.

A mechanism that describes the action of surfactants was proposed. Surfactant molecules are able to sorb to surfaces so that their hydrophobic tails adsorb to the surfaces, while their hydrophilic heads remain in the aqueous phase. It would appear that the ability of a surfactant to inhibit adhesion is the result of the formation of this film, which makes hydrophobic surfaces become more hydrophilic. Because the microorganisms that are more likely to adhere to hydrophobic surfaces are more hydrophobic themselves, the increased hydrophilicity of the surface decreases the affinity of these microorganisms for these surfaces. Thus, the presence of surfactant would inhibit the microorganisms from adhering to such surfaces or cause the release of previously adhering microorganisms from such surfaces.

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APPENDIX A - Raw Data

Table A-1: Surface Tension (dynes/cm) at 22.0°C of Triton X-100 Solutions for the Determination of its Critical Micelle Concentration

(a) in the Absence of Organic Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	39.3	39.2	39.3
0.14	38.3	38.2	38.3
0.16	37.3	37.4	37.4
0.18	36.4	36.3	36.4
0.20	35.4	35.4	35.4
0.22	34.2	34.3	34.3
0.24	33.3	33.2	33.3
0.26	33.2	33.3	33.3
0.28	33.3	33.3	33.3
0.30	33.2	33.3	33.3

(b) in the Presence of the EDM Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	39.3	39.3	39.3
0.14	38.6	38.7	38.7
0.16	38.2	38.2	38.2
0.18	37.8	37.8	37.8
0.20	37.5	37.4	37.5
0.22	36.8	36.9	36.9
0.24	36.3	36.2	36.3
0.26	36.3	36.3	36.3
0.28	36.3	36.3	36.3
0.30	36.2	36.3	36.3

(c) in the Presence of the PAA Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	40.5	40.4	40.5
0.14	40.0	40.0	40.0
0.16	39.6	39.6	39.6
0.18	39.2	39.1	39.2
0.20	38.7	38.6	38.7
0.22	38.1	38.1	38.1
0.24	37.5	37.5	37.5
0.26	37.5	37.5	37.5
0.28	37.4	37.5	37.5
0.30	37.5	37.5	37.5

(d) in the Presence of DEV Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	39.7	39.6	39.7
0.14	38.8	38.8	38.8
0.16	38.1	38.2	38.2
0.18	37.6	37.5	37.6
0.20	37.0	37.1	37.1
0.22	36.4	36.4	36.4
0.24	35.9	35.8	35.9
0.26	35.8	35.7	35.8
0.28	35.8	35.8	35.8
0.30	35.8	35.8	35.8

(e) in the Presence of the MTL Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	39.3	39.2	39.3
0.14	38.3	38.2	38.3
0.16	37.3	37.4	37.4
0.18	36.4	36.3	36.4
0.20	35.4	35.4	35.4
0.22	34.2	34.3	34.3
0.24	33.3	33.2	33.3
0.26	33.2	33.3	33.3
0.28	33.3	33.3	33.3
0.30	33.2	33.3	33.3

Table A-2: Surface Tension (dynes/cm) at 22.9°C of Triton X-100 Solutions for the Determination of its Critical Micelle Concentration in the Presence and Absence of the *Rhodococcus* Species

(a) in the Presence of Microorganisms

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	42.2	42.2	42.2
0.14	41.2	41.2	41.2
0.16	40.2	40.1	40.2
0.18	39.2	39.2	39.2
0.20	38.2	38.3	38.3
0.22	37.3	37.2	37.3
0.24	36.2	36.2	36.2
0.26	36.1	36.2	36.2
0.28	36.2	36.2	36.2
0.30	36.2	36.2	36.2

(b) in the Absence of Microorganisms

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.12	41.3	41.3	41.3
0.14	40.3	40.3	40.3
0.16	39.3	39.4	39.4
0.18	38.3	38.4	38.4
0.20	37.4	37.4	37.4
0.22	36.5	36.4	36.5
0.24	35.5	35.4	35.5
0.26	35.5	35.5	35.5
0.28	35.4	35.5	35.5
0.30	35.5	35.5	35.5

Table A-3: Surface Tension (dynes/cm) at 21.8°C of Dowfax 8390 Solutions for the Determination of its Critical Micelle Concentration

(a) in the Absence of Organic Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	52.7	52.6	52.7
0.3	52.0	52.0	52.0
0.4	51.6	51.5	51.6
0.5	51.2	51.1	51.2
0.6	50.9	50.8	50.9
0.7	50.6	50.6	50.6
0.8	50.3	50.4	50.4
0.9	50.4	50.4	50.4
1.0	50.4	50.5	50.5
1.1	50.4	50.4	50.4

(b) in the Presence of the EDM Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	43.7	43.6	43.7
0.3	43.3	43.2	43.3
0.4	42.8	42.9	42.9
0.5	42.7	42.6	42.7
0.6	42.5	42.5	42.5
0.7	42.3	42.3	42.3
0.8	42.0	42.0	42.0
0.9	42.1	42.0	42.1
1.0	42.0	42.0	42.0
1.1	42.0	41.9	42.0

(c) in the Presence of the PAA Tar

Surfactant Concentration (mM)	Surface Tesnion (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	44.5	44.6	44.6
0.3	44.2	44.2	44.2
0.4	44.0	43.9	44.0
0.5	43.8	43.7	43.8
0.6	43.5	43.6	43.6
0.7	43.3	43.2	43.3
0.8	43.0	43.0	43.0
0.9	43.0	42.9	43.0
1.0	43.0	43.0	43.0
1.1	43.0	43.0	43.0

(d) in the Presence of the DEV Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	43.7	43.6	43.7
0.3	43.5	43.5	43.5
0.4	43.3	43.3	43.3
0.5	43.0	43.1	43.1
0.6	42.9	42.9	42.9
0.7	42.5	42.6	42.6
0.8	42.2	42.2	42.2
0.9	42.2	42.2	42.2
1.0	42.2	42.2	42.2
1.1	42.2	42.2	42.2

(e) in the Presence of the MTL Tar

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	46.0	46.0	46.0
0.3	45.6	45.5	45.6
0.4	45.2	45.1	45.2
0.5	45.0	44.9	45.0
0.6	44.8	44.8	44.8
0.7	44.5	44.5	44.5
0.8	44.2	44.2	44.2
0.9	44.2	44.2	44.2
1.0	44.1	44.2	44.2
1.1	44.2	44.1	44.2

Table A-4: Surface Tension (dynes/cm) at 22.9°C of Dowfax 8390 Solutions for the Determination of its Critical Micelle Concentration in the Presence and Absence of the *Rhodococcus* Species

(a) in the Presence of Microorganisms

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	52.0	51.9	52.0
0.3	51.6	51.5	51.6
0.4	51.3	51.3	51.3
0.5	51.0	51.1	51.1
0.6	50.7	50.6	50.7
0.7	50.3	50.3	50.3
0.8	49.8	49.9	49.9
0.9	49.9	49.9	49.9
1.0	49.9	49.8	49.9
1.1	49.9	49.9	49.9

(b) in the Absence of Microorganisms

Surfactant Concentration (mM)	Surface Tension (Run 1)	Surface Tension (Run 2)	Average Surface Tension
0.2	53.7	53.6	53.7
0.3	53.0	53.0	53.0
0.4	52.5	52.6	52.6
0.5	52.2	52.2	52.2
0.6	52.0	52.0	52.0
0.7	51.7	51.6	51.7
0.8	51.4	51.3	51.4
0.9	51.4	51.4	51.4
1.0	51.4	51.4	51.4
1.1	51.4	51.4	51.4

Table A-5: OD_{600} Readings for the Determination of the Initial Adhesion of *Rhodococcus* Cells to Organic Tars (Series 1)

Test Tube Contents	Tar	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Tube 4 O.D.	Tube 5 O.D.	Tube 6 O.D.	Average O.D.
Cells	EDM	0.333	0.351	0.363	0.370	0.368	0.365	0.358
Cells + Triton X-100	EDM	0.560	0.606	0.575				0.580
Cells + Dowfax 8390	EDM	0.433	0.440	0.452				0.442
Buffer	EDM	0.005	0.008	0.008	0.014	0.009	0.012	0.009
Buffer +Triton X-100	EDM	0.104	0.078	0.086				0.089
Buffer + Dowfax 8390	EDM	0.078	0.055	0.062				0.065
Cells	PAA	0.321	0.342	0.334	0.351	0.341	0.366	0.343
Cells + Triton X-100	PAA	1.275	1.323	1.510				1.369
Cells + Dowfax 8390	PAA	0.827	0.768	0.676				0.757
Buffer	PAA	0.007	0.012	0.018	0.014	0.014	0.006	0.012
Buffer +Triton X-100	PAA	0.817	0.805	0.823				0.815
Buffer + Dowfax 8390	PAA	0.201	0.261	0.237				0.233
Cells	DEV	0.341	0.353	0.366	0.359	0.371	0.356	0.358
Cells + Triton X-100	DEV	1.091	1.266	1.175				1.177
Cells + Dowfax 8390	DEV	0.404	0.424	0.429				0.419
Buffer	DEV	0.014	0.010	0.011	0.007	0.014	0.013	0.012
Buffer +Triton X-100	DEV	0.578	0.640	0.648				0.622
Buffer + Dowfax 8390	DEV	0.043	0.019	0.035				0.032
Cells	MTL	0.349	0.378	0.365	0.369	0.356	0.339	0.359
Cells + Triton X-100	MTL	0.578	0.571	0.564				0.571
Cells + Dowfax 8390	MTL	0.450	0.445	0.452				0.449
Buffer	MTL	0.000	0.000	-0.001	-0.002	0.001	0.000	-0.000
Buffer +Triton X-100	MTL	0.008	0.005	0.009				0.007
Buffer + Dowfax 8390	MTL	0.004	0.005	0.006				0.005
Cells	NONE	0.294	0.318	0.296	0.286	0.287	0.323	0.301
Cells + Triton X-100	NONE	0.567	0.554	0.565				0.562
Cells + Dowfax 8390	NONE	0.473	0.462	0.483				0.473

Table A-6: OD$_{600}$ Readings for the Determination of the Initial Adhesion of *Rhodococcus* Cells to Organic Tars (Series 2)

Test Tube Contents	Tar	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Tube 4 O.D.	Tube 5 O.D.	Tube 6 O.D.	Average O.D.
Cells	EDM	0.370	0.355	0.382	0.367	0.358	0.371	0.367
Cells + Triton X-100	EDM	0.595	0.578	0.585				0.586
Cells + Dowfax 8390	EDM	0.439	0.451	0.464				0.451
Buffer	EDM	0.017	0.016	0.010	0.012	0.018	0.010	0.014
Buffer +Triton X-100	EDM	0.098	0.094	0.101				0.098
Buffer + Dowfax 8390	EDM	0.038	0.027	0.022				0.029
Cells	PAA	0.383	0.367	0.391	0.372	0.377	0.381	0.379
Cells + Triton X-100	PAA	1.361	1.538	1.285				1.395
Cells + Dowfax 8390	PAA	0.798	0.869	0.824				0.830
Buffer	PAA	0.014	0.009	0.010	0.008	0.011	0.013	0.011
Buffer +Triton X-100	PAA	0.809	0.827	0.801				0.812
Buffer + Dowfax 8390	PAA	0.258	0.267	0.252				0.259
Cells	DEV	0.353	0.342	0.374	0.361	0.363	0.356	0.358
Cells + Triton X-100	DEV	1.207	1.154	1.092				1.151
Cells + Dowfax 8390	DEV	0.448	0.462	0.457				0.456
Buffer	DEV	0.007	0.009	0.006	0.009	0.011	0.006	0.008
Buffer +Triton X-100	DEV	0.592	0.601	0.577				0.590
Buffer + Dowfax 8390	DEV	0.020	0.024	0.019				0.021
Cells	MTL	0.378	0.372	0.379	0.381	0.375	0.375	0.377
Cells + Triton X-100	MTL	0.589	0.594	0.592				0.592
Cells + Dowfax 8390	MTL	0.494	0.497	0.503				0.498
Buffer	MTL	-0.001	0.001	0.000	0.000	-0.002	0.001	-0.000
Buffer +Triton X-100	MTL	0.004	0.006	0.007				0.006
Buffer + Dowfax 8390	MTL	0.003	0.001	0.003				0.002
Cells	NONE	0.284	0.268	0.292	0.291	0.288	0.301	0.287
Cells + Triton X-100	NONE	0.594	0.596	0.597				0.596
Cells + Dowfax 8390	NONE	0.490	0.493	0.475				0.486

Table A-7: OD_{600} Readings for the Determination of the Initial Adhesion of *Pseudomonas* Cells to Organic Tars (Series 1)

Test Tube Contents	Tar	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	0.284	0.266	0.275	0.275
Cells + Triton X-100	EDM	0.586	0.603	0.617	0.602
Cells + Dowfax 8390	EDM	0.585	0.565	0.581	0.577
Buffer	EDM	-0.001	0.003	0.005	0.002
Buffer +Triton X-100	EDM	0.091	0.087	0.096	0.091
Buffer + Dowfax 8390	EDM	0.031	0.018	0.027	0.025
Cells	PAA	0.287	0.294	0.290	0.290
Cells + Triton X-100	PAA	1.244	1.237	1.216	1.232
Cells + Dowfax 8390	PAA	0.748	0.755	0.737	0.747
Buffer	PAA	0.001	0.004	0.001	0.002
Buffer +Triton X-100	PAA	0.794	0.802	0.831	0.809
Buffer + Dowfax 8390	PAA	0.257	0.231	0.234	0.241
Cells	DEV	0.346	0.337	0.340	0.341
Cells + Triton X-100	DEV	1.034	1.062	1.054	1.050
Cells + Dowfax 8390	DEV	0.520	0.531	0.513	0.521
Buffer	DEV	0.011	0.008	0.012	0.010
Buffer +Triton X-100	DEV	0.609	0.590	0.617	0.605
Buffer + Dowfax 8390	DEV	0.018	0.026	0.024	0.023
Cells	MTL	0.371	0.362	0.355	0.363
Cells + Triton X-100	MTL	0.480	0.491	0.494	0.488
Cells + Dowfax 8390	MTL	0.574	0.583	0.567	0.575
Buffer	MTL	-0.001	0.000	0.001	0.000
Buffer +Triton X-100	MTL	0.002	0.003	0.006	0.004
Buffer + Dowfax 8390	MTL	0.000	-0.001	0.002	0.000
Cells	NONE	0.361	0.353	0.342	0.352
Cells + Triton X-100	NONE	0.502	0.494	0.496	0.497
Cells + Dowfax 8390	NONE	0.532	0.521	0.527	0.527

Table A-8: OD$_{600}$ Readings for the Determination of the Initial Adhesion of *Pseudomonas* Cells to Organic Tars (Series 2)

Test Tube Contents	Tar	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	0.278	0.269	0.284	0.277
Cells + Triton X-100	EDM	0.613	0.607	0.591	0.604
Cells + Dowfax 8390	EDM	0.564	0.587	0.578	0.576
Buffer	EDM	0.005	0.003	0.001	0.003
Buffer +Triton X-100	EDM	0.090	0.094	0.104	0.096
Buffer + Dowfax 8390	EDM	0.031	0.037	0.019	0.029
Cells	PAA	0.305	0.297	0.286	0.296
Cells + Triton X-100	PAA	1.248	1.236	1.204	1.229
Cells + Dowfax 8390	PAA	0.718	0.771	0.740	0.743
Buffer	PAA	0.002	0.004	0.001	0.002
Buffer +Triton X-100	PAA	0.806	0.815	0.801	0.807
Buffer + Dowfax 8390	PAA	0.252	0.231	0.266	0.250
Cells	DEV	0.356	0.360	0.371	0.362
Cells + Triton X-100	DEV	1.081	1.063	1.070	1.071
Cells + Dowfax 8390	DEV	0.523	0.529	0.540	0.531
Buffer	DEV	0.010	0.007	0.006	0.008
Buffer +Triton X-100	DEV	0.607	0.599	0.612	0.606
Buffer + Dowfax 8390	DEV	0.024	0.040	0.036	0.033
Cells	MTL	0.354	0.369	0.371	0.365
Cells + Triton X-100	MTL	0.505	0.496	0.491	0.497
Cells + Dowfax 8390	MTL	0.572	0.579	0.563	0.571
Buffer	MTL	0.000	0.001	-0.001	0.000
Buffer +Triton X-100	MTL	0.003	0.002	0.006	0.004
Buffer + Dowfax 8390	MTL	0.000	-0.001	0.002	0.000
Cells	NONE	0.392	0.381	0.376	0.383
Cells + Triton X-100	NONE	0.513	0.518	0.527	0.519
Cells + Dowfax 8390	NONE	0.547	0.549	0.536	0.544

Table A-9: OD$_{600}$ Readings for the Determination of the Removal of *Rhodococcus* Cells from Organic Tars (Series 1)

Test Tube Contents	Tar	Solution Added	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	None	0.414	0.429	0.410	0.418
Cells	EDM	Triton X-100	0.842	0.811	0.829	0.827
Cells	EDM	Dowfax 8390	0.472	0.451	0.443	0.455
Buffer	EDM	None	-0.001	0.003	0.001	0.001
Buffer	EDM	Triton X-100	0.327	0.312	0.306	0.315
Buffer	EDM	Dowfax 8390	0.005	0.008	0.006	0.006
Cells	PAA	None	0.301	0.292	0.295	0.296
Cells	PAA	Triton X-100	1.082	1.029	1.041	1.051
Cells	PAA	Dowfax 8390	0.612	0.646	0.630	0.629
Buffer	PAA	None	0.002	0.000	-0.001	0.000
Buffer	PAA	Triton X-100	0.523	0.492	0.504	0.506
Buffer	PAA	Dowfax 8390	0.036	0.042	0.047	0.042
Cells	DEV	None	0.286	0.304	0.323	0.304
Cells	DEV	Triton X-100	0.680	0.701	0.714	0.698
Cells	DEV	Dowfax 8390	0.581	0.565	0.584	0.577
Buffer	DEV	None	0.007	0.004	-0.002	0.003
Buffer	DEV	Triton X-100	0.277	0.284	0.263	0.275
Buffer	DEV	Dowfax 8390	0.069	0.087	0.092	0.083
Cells	MTL	None	0.401	0.398	0.403	0.401
Cells	MTL	Triton X-100	0.582	0.573	0.589	0.581
Cells	MTL	Dowfax 8390	0.552	0.561	0.567	0.560
Buffer	MTL	None	0.000	-0.001	0.000	-0.000
Buffer	MTL	Triton X-100	-0.002	0.001	-0.001	-0.001
Buffer	MTL	Dowfax 8390	0.001	0.000	-0.001	0.000
Cells	NONE	None	0.294	0.303	0.275	0.291
Cells	NONE	Triton X-100	0.554	0.586	0.571	0.570
Cells	NONE	Dowfax 8390	0.598	0.583	0.586	0.589

Table A-10: OD_{600} Readings for the Determination of the Removal of *Rhodococcus* Cells from Organic Tars (Series 2)

Test Tube Contents	Tar	Solution Added	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	None	0.420	0.425	0.419	0.421
Cells	EDM	Triton X-100	0.829	0.834	0.835	0.833
Cells	EDM	Dowfax 8390	0.448	0.453	0.455	0.452
Buffer	EDM	None	0.001	0.002	0.000	0.001
Buffer	EDM	Triton X-100	0.321	0.316	0.314	0.317
Buffer	EDM	Dowfax 8390	0.008	0.005	0.008	0.007
Cells	PAA	None	0.304	0.301	0.296	0.300
Cells	PAA	Triton X-100	1.052	1.061	1.082	1.065
Cells	PAA	Dowfax 8390	0.634	0.626	0.621	0.627
Buffer	PAA	None	0.001	0.002	-0.001	0.001
Buffer	PAA	Triton X-100	0.493	0.487	0.498	0.493
Buffer	PAA	Dowfax 8390	0.045	0.047	0.051	0.048
Cells	DEV	None	0.302	0.306	0.295	0.301
Cells	DEV	Triton X-100	0.700	0.695	0.687	0.694
Cells	DEV	Dowfax 8390	0.582	0.573	0.578	0.578
Buffer	DEV	None	0.004	0.002	0.005	0.004
Buffer	DEV	Triton X-100	0.281	0.274	0.279	0.278
Buffer	DEV	Dowfax 8390	0.081	0.083	0.075	0.080
Cells	MTL	None	0.396	0.402	0.395	0.398
Cells	MTL	Triton X-100	0.587	0.579	0.581	0.582
Cells	MTL	Dowfax 8390	0.565	0.560	0.557	0.561
Buffer	MTL	None	0.000	-0.001	0.001	0.000
Buffer	MTL	Triton X-100	0.000	0.000	0.001	0.000
Buffer	MTL	Dowfax 8390	0.001	-0.002	0.001	0.000
Cells	NONE	None	0.298	0.290	0.286	0.291
Cells	NONE	Triton X-100	0.574	0.562	0.564	0.567
Cells	NONE	Dowfax 8390	0.582	0.591	0.587	0.587

Table A-11: OD$_{600}$ Readings for the Determination of the Removal of *Pseudomonas* Cells from Organic Tars (Series 1)

Test Tube Contents	Tar	Solution Added	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	None	0.285	0.296	0.282	0.288
Cells	EDM	Triton X-100	0.782	0.789	0.756	0.776
Cells	EDM	Dowfax 8390	0.513	0.506	0.523	0.514
Buffer	EDM	None	0.000	0.002	0.001	0.001
Buffer	EDM	Triton X-100	0.310	0.363	0.331	0.335
Buffer	EDM	Dowfax 8390	0.006	0.002	0.000	0.003
Cells	PAA	None	0.303	0.300	0.294	0.299
Cells	PAA	Triton X-100	0.887	0.902	0.911	0.900
Cells	PAA	Dowfax 8390	0.432	0.450	0.454	0.445
Buffer	PAA	None	0.001	0.002	-0.001	0.001
Buffer	PAA	Triton X-100	0.504	0.514	0.520	0.513
Buffer	PAA	Dowfax 8390	0.027	0.047	0.036	0.037
Cells	DEV	None	0.347	0.329	0.342	0.339
Cells	DEV	Triton X-100	0.803	0.778	0.782	0.788
Cells	DEV	Dowfax 8390	0.421	0.405	0.394	0.407
Buffer	DEV	None	0.001	0.003	0.000	0.001
Buffer	DEV	Triton X-100	0.291	0.267	0.281	0.280
Buffer	DEV	Dowfax 8390	0.094	0.077	0.083	0.085
Cells	MTL	None	0.360	0.384	0.379	0.374
Cells	MTL	Triton X-100	0.381	0.382	0.387	0.383
Cells	MTL	Dowfax 8390	0.392	0.374	0.386	0.384
Buffer	MTL	None	-0.001	0.000	0.002	0.000
Buffer	MTL	Triton X-100	0.000	-0.002	0.003	0.000
Buffer	MTL	Dowfax 8390	0.001	0.000	0.003	0.001
Cells	NONE	None	0.402	0.388	0.391	0.394
Cells	NONE	Triton X-100	0.504	0.513	0.493	0.503
Cells	NONE	Dowfax 8390	0.485	0.470	0.471	0.475

Table A-12: OD_{600} Readings for the Determination of the Removal of *Pseudomonas* Cells from Organic Tars (Series 2)

Test Tube Contents	Tar	Solution Added	Tube 1 O.D.	Tube 2 O.D.	Tube 3 O.D.	Average O.D.
Cells	EDM	None	0.291	0.296	0.301	0.296
Cells	EDM	Triton X-100	0.790	0.787	0.771	0.783
Cells	EDM	Dowfax 8390	0.534	0.541	0.517	0.531
Buffer	EDM	None	0.000	0.002	-0.001	0.000
Buffer	EDM	Triton X-100	0.305	0.325	0.337	0.322
Buffer	EDM	Dowfax 8390	0.005	0.002	0.003	0.003
Cells	PAA	None	0.311	0.306	0.315	0.311
Cells	PAA	Triton X-100	0.915	0.907	0.902	0.908
Cells	PAA	Dowfax 8390	0.456	0.467	0.468	0.464
Buffer	PAA	None	0.001	0.000	0.003	0.001
Buffer	PAA	Triton X-100	0.502	0.496	0.498	0.499
Buffer	PAA	Dowfax 8390	0.031	0.036	0.027	0.031
Cells	DEV	None	0.341	0.338	0.344	0.341
Cells	DEV	Triton X-100	0.801	0.811	0.798	0.803
Cells	DEV	Dowfax 8390	0.412	0.431	0.426	0.423
Buffer	DEV	None	0.001	0.000	0.001	0.001
Buffer	DEV	Triton X-100	0.290	0.278	0.282	0.283
Buffer	DEV	Dowfax 8390	0.092	0.084	0.094	0.090
Cells	MTL	None	0.391	0.384	0.378	0.384
Cells	MTL	Triton X-100	0.388	0.391	0.392	0.390
Cells	MTL	Dowfax 8390	0.372	0.399	0.386	0.386
Buffer	MTL	None	0.000	-0.002	0.001	-0.000
Buffer	MTL	Triton X-100	0.001	0.003	0.000	0.001
Buffer	MTL	Dowfax 8390	-0.001	0.000	0.002	0.000
Cells	NONE	None	0.407	0.396	0.411	0.405
Cells	NONE	Triton X-100	0.522	0.515	0.508	0.515
Cells	NONE	Dowfax 8390	0.482	0.491	0.480	0.484

Table A-13: OD$_{600}$ Readings for the Determination of the Adhesion of the *Rhodococcus* Species to Solid Anthracene for the Purpose of Microbial Growth

(a) in the Absence of Surfactant

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Average O.D.
0	0.117	0.125	0.125	0.116	0.121
1	0.228	0.237	0.246	0.248	0.240
2	0.366	0.342	0.351	0.355	0.354
3	0.647	0.659	0.670	0.672	0.662
5	0.902	0.913	0.921	0.929	0.916
7	1.116	1.163	1.178	1.198	1.164
10	1.021	0.987	1.002	0.993	1.001
14	0.961	0.948	0.954	0.930	0.948

(b) in the Presence of Triton X-100

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Flask 5 O.D.	Average O.D.
0	0.191	0.183	0.165	0.142	0.164	0.169
1	0.286	0.289	0.288	0.305	0.303	0.294
2	0.361	0.328	0.357	0.350	0.342	0.348
3	0.426	0.418	0.415	0.430	0.411	0.420
5	0.551	0.506	0.502	0.559	0.511	0.526
7	0.682	0.670	0.665	0.690	0.659	0.673
10	0.816	0.805	0.803	0.821	0.800	0.809
14	0.821	0.809	0.827	0.817	0.806	0.816

(c) in the Presence of Dowfax 8390

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Average O.D.
0	0.156	0.142	0.144	0.144	0.147
1	0.233	0.212	0.207	0.201	0.213
2	0.278	0.256	0.269	0.272	0.269
3	0.297	0.275	0.282	0.298	0.288
5	0.301	0.291	0.297	0.307	0.299
7	0.307	0.306	0.312	0.313	0.310
10	0.320	0.308	0.317	0.321	0.317
14	0.314	0.291	0.299	0.304	0.302

Table A-14: OD$_{600}$ Readings for the Determination of the Adhesion of the *Pseudomonas* Species to Solid Anthracene for the Purpose of Microbial Growth

(a) in the Absence of Surfactant

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Flask 5 O.D.	Average O.D.
0	0.056	0.055	0.053	0.057	0.060	0.056
1	0.183	0.204	0.198	0.166	0.175	0.185
2	0.234	0.258	0.239	0.247	0.251	0.246
3	0.270	0.305	0.279	0.291	0.293	0.288
5	0.291	0.317	0.302	0.316	0.325	0.310
7	0.325	0.341	0.337	0.350	0.353	0.341
10	0.305	0.327	0.318	0.331	0.332	0.323
14	0.298	0.309	0.304	0.313	0.315	0.308

(b) in the Presence of Triton X-100

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Average O.D.
0	0.065	0.067	0.065	0.066	0.066
1	0.071	0.079	0.071	0.077	0.075
2	0.080	0.097	0.081	0.090	0.087
3	0.090	0.112	0.100	0.105	0.102
5	0.104	0.123	0.109	0.131	0.117
7	0.113	0.153	0.117	0.156	0.135
10	0.114	0.138	0.126	0.139	0.129
14	0.107	0.123	0.116	0.120	0.117

(c) in the Presence of Dowfax 8390

Time (days)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Average O.D.
0	0.055	0.061	0.058	0.062	0.059
1	0.057	0.062	0.065	0.071	0.064
2	0.059	0.081	0.077	0.083	0.075
3	0.068	0.101	0.084	0.089	0.086
5	0.080	0.096	0.090	0.092	0.090
7	0.091	0.089	0.096	0.094	0.093
10	0.088	0.075	0.078	0.097	0.085
14	0.082	0.071	0.076	0.088	0.079

Table A-15: OD$_{600}$ Readings for the Determination of the Adhesion of the *Pseudomonas* Species to Dissolved Glucose for the Purpose of Microbial Growth

(a) in the Absence of Surfactant

Time (hours)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Flask 5 O.D.	Average O.D.
0	0.091	0.094	0.092	0.090	0.093	0.092
2	0.143	0.148	0.148	0.146	0.150	0.147
4	0.264	0.267	0.269	0.267	0.270	0.267
6	0.508	0.504	0.520	0.521	0.513	0.513
8	0.986	0.991	1.002	0.983	0.987	0.990
12	0.868	0.882	0.880	0.872	0.888	0.878
24	0.775	0.787	0.796	0.786	0.792	0.787

(b) in the Presence of Triton X-100

Time (hours)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Flask 5 O.D.	Average O.D.
0	0.091	0.093	0.090	0.093	0.093	0.092
2	0.158	0.165	0.162	0.158	0.162	0.161
4	0.294	0.284	0.286	0.278	0.288	0.286
6	0.583	0.591	0.595	0.578	0.600	0.589
8	0.956	0.942	0.936	0.937	0.930	0.940
12	0.851	0.847	0.829	0.840	0.838	0.841
24	0.780	0.768	0.752	0.763	0.765	0.766

(c) in the Presence of Dowfax 8390

Time (hours)	Flask 1 O.D.	Flask 2 O.D.	Flask 3 O.D.	Flask 4 O.D.	Flask 5 O.D.	Average O.D.
0	0.089	0.088	0.092	0.091	0.094	0.091
2	0.155	0.159	0.165	0.163	0.156	0.160
4	0.281	0.283	0.284	0.280	0.278	0.281
6	0.567	0.558	0.571	0.572	0.561	0.566
8	0.997	0.971	1.077	1.012	1.017	1.015
12	0.833	0.826	0.841	0.822	0.831	0.831
24	0.718	0.726	0.747	0.734	0.739	0.733

Table A-16: Total *Rhodococcus* Cells Recovered from Column (Series 1)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.192	0.9	0.333	1.6	0.671	3.3
4	0.096	1.4	0.214	2.6	0.533	5.9
6	0.093	1.8	0.208	3.6	0.518	8.4
8	0.087	2.3	0.203	4.6	0.488	10.8
10	0.100	2.8	0.191	5.5	0.483	13.1
12	0.142	3.4	0.196	6.4	0.476	15.4
14	0.188	4.4	0.202	7.4	0.472	17.7
16	0.232	5.5	0.211	8.4	0.467	20.0
18	0.245	6.7	0.225	9.5	0.463	22.3
20	0.258	7.9	0.235	10.6	0.458	24.5
22	0.277	9.3	0.247	11.8	0.456	26.7
24	0.289	10.7	0.264	13.1	0.452	28.9
26	0.315	12.2	0.279	14.4	0.449	31.1
28	0.332	13.8	0.285	15.8	0.441	33.3
30	0.351	15.5	0.296	17.2	0.435	35.4
32	0.375	17.3	0.301	18.6	0.429	37.5
34	0.362	19.1	0.310	20.1	0.423	39.5
36	0.347	20.8	0.317	21.6	0.410	41.5
38	0.329	22.4	0.326	23.2	0.397	43.5
40	0.314	23.9	0.334	24.8	0.386	45.4
42	0.297	25.3	0.349	26.5	0.378	47.2
44	0.283	26.7	0.358	28.2	0.367	49.0
46	0.263	28.0	0.367	29.9	0.359	50.7
48	0.248	29.2	0.382	31.8	0.351	52.4
50	0.234	30.3	0.391	33.6	0.344	54.1
52	0.214	31.4	0.434	35.7	0.338	55.8
54	0.176	32.2	0.396	37.6	0.262	57.0
56	0.129	32.8	0.227	38.7	0.135	57.7
58	0.058	33.1	0.122	39.3	0.072	58.1
60	0.032	33.3	0.061	39.6	0.043	58.3
62	0.018	33.4	0.029	39.7	0.027	58.4

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.687**
- **in the presence of Triton X-100: 0.696**
- **in the presence of Dowfax 8390: 0.684**

Table A-17: Total *Rhodococcus* Cells Recovered from Column (Series 2)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.186	0.9	0.327	1.6	0.658	3.2
4	0.091	1.4	0.227	2.7	0.529	5.8
6	0.088	1.8	0.221	3.8	0.520	8.3
8	0.086	2.2	0.217	4.9	0.487	10.7
10	0.095	2.7	0.215	6.0	0.483	13.1
12	0.137	3.3	0.218	7.1	0.475	15.4
14	0.180	4.2	0.224	8.2	0.472	17.7
16	0.225	5.3	0.229	9.3	0.469	20.0
18	0.241	6.5	0.240	10.5	0.466	22.3
20	0.250	7.7	0.248	11.7	0.460	24.5
22	0.269	9.1	0.255	13.0	0.457	26.8
24	0.282	10.4	0.263	14.3	0.452	29.0
26	0.308	12.0	0.274	15.7	0.447	31.2
28	0.327	13.6	0.281	17.1	0.440	33.3
30	0.341	15.2	0.290	18.5	0.431	35.4
32	0.368	17.0	0.295	20.0	0.425	37.5
34	0.357	18.8	0.299	21.4	0.417	39.5
36	0.344	20.5	0.306	23.0	0.408	41.5
38	0.321	22.0	0.314	24.5	0.391	43.4
40	0.309	23.6	0.321	26.1	0.379	45.3
42	0.295	25.0	0.330	27.7	0.370	47.1
44	0.281	26.4	0.338	29.4	0.362	48.9
46	0.262	27.7	0.345	31.1	0.353	50.6
48	0.248	28.9	0.354	32.9	0.346	52.3
50	0.236	30.0	0.363	34.7	0.339	53.9
52	0.216	31.1	0.387	36.6	0.333	55.6
54	0.179	32.0	0.375	38.5	0.251	56.8
56	0.133	32.6	0.194	39.4	0.129	57.4
58	0.062	32.9	0.107	40.0	0.068	57.8
60	0.034	33.1	0.054	40.2	0.039	57.9
62	0.019	33.2	0.028	40.4	0.024	58.1

Initial Optical Densities of Microbial Suspensions:

in the absence of surfactant:	**0.680**
in the presence of Triton X-100:	**0.672**
in the presence of Dowfax 8390:	**0.682**

Table A-18: Total *Rhodococcus* Cells Recovered from Column (Series 3)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.209	1.1	0.326	1.7	0.640	3.3
4	0.108	1.6	0.221	2.8	0.507	5.8
6	0.103	2.1	0.215	3.9	0.492	8.4
8	0.095	2.6	0.211	4.9	0.453	10.7
10	0.099	3.1	0.207	6.0	0.450	13.0
12	0.132	3.8	0.210	7.1	0.445	15.2
14	0.176	4.6	0.214	8.1	0.441	17.5
16	0.220	5.8	0.222	9.3	0.438	19.7
18	0.233	6.9	0.234	10.5	0.435	21.9
20	0.246	8.2	0.245	11.7	0.432	24.1
22	0.264	9.5	0.255	13.0	0.429	26.3
24	0.275	10.9	0.266	14.3	0.425	28.5
26	0.303	12.4	0.276	15.7	0.423	30.6
28	0.317	14.0	0.282	17.2	0.419	32.8
30	0.335	15.7	0.291	18.6	0.417	34.9
32	0.358	17.5	0.298	20.2	0.414	37.0
34	0.347	19.3	0.307	21.7	0.408	39.1
36	0.336	21.0	0.315	23.3	0.393	41.1
38	0.318	22.6	0.324	25.0	0.382	43.0
40	0.306	24.1	0.331	26.6	0.371	44.9
42	0.286	25.5	0.343	28.4	0.362	46.8
44	0.277	26.9	0.352	30.2	0.351	48.6
46	0.250	28.2	0.361	32.0	0.343	50.3
48	0.238	29.4	0.375	33.9	0.336	52.0
50	0.229	30.6	0.388	35.9	0.328	53.7
52	0.211	31.6	0.425	38.0	0.321	55.3
54	0.166	32.5	0.393	40.0	0.251	56.6
56	0.119	33.1	0.196	41.0	0.145	57.3
58	0.060	33.4	0.102	41.5	0.078	57.7
60	0.033	33.5	0.053	41.8	0.047	58.0
62	0.016	33.6	0.028	41.9	0.029	58.1

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.661**
- **in the presence of Triton X-100: 0.657**
- **in the presence of Dowfax 8390: 0.654**

Table A-19: Total *Rhodococcus* Cells Recovered from Column (Reduced Loading)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.178	1.4	0.316	2.5	0.618	4.9
4	0.090	2.1	0.207	4.1	0.481	8.7
6	0.087	2.8	0.186	5.6	0.453	12.2
8	0.081	3.4	0.183	7.0	0.448	15.7
10	0.092	4.1	0.177	8.4	0.444	19.2
12	0.129	5.1	0.181	9.8	0.438	22.7
14	0.174	6.5	0.188	11.3	0.430	26.1
16	0.207	8.1	0.193	12.8	0.425	29.4
18	0.219	9.8	0.209	14.4	0.418	32.7
20	0.231	11.6	0.218	16.1	0.409	35.9
22	0.248	13.5	0.229	17.9	0.399	39.1
24	0.255	15.5	0.243	19.8	0.390	42.1
26	0.271	17.6	0.258	21.8	0.376	45.1
28	0.290	19.9	0.263	23.9	0.361	48.0
30	0.312	22.3	0.270	26.0	0.337	50.6
32	0.339	24.9	0.277	28.2	0.291	52.9
34	0.196	26.5	0.269	30.3	0.156	54.1
36	0.126	27.5	0.138	31.4	0.077	54.7
38	0.065	28.0	0.068	31.9	0.039	55.0
40	0.034	28.2	0.035	32.2	0.023	55.2
42	0.019	28.4	0.016	32.3	0.014	55.3

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.642**
- **in the presence of Triton X-100: 0.638**
- **in the presence of Dowfax 8390: 0.635**

Table A-20: Total *Pseudomonas* Cells Recovered from Column (Series 1)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.785	3.6	0.759	3.4	0.752	3.4
4	0.742	6.9	0.739	6.8	0.730	6.7
6	0.739	10.3	0.732	10.1	0.726	9.9
8	0.738	13.7	0.731	13.4	0.719	13.2
10	0.735	17.0	0.731	16.7	0.710	16.4
12	0.734	20.3	0.730	20.0	0.701	19.5
14	0.730	23.7	0.730	23.3	0.699	22.7
16	0.729	27.0	0.729	26.6	0.698	25.8
18	0.728	30.3	0.728	29.9	0.696	28.9
20	0.728	33.6	0.726	33.2	0.696	32.1
22	0.727	36.9	0.725	36.5	0.693	35.2
24	0.726	40.2	0.725	39.8	0.692	38.3
26	0.726	43.5	0.724	43.1	0.691	41.4
28	0.727	46.8	0.725	46.3	0.688	44.5
30	0.726	50.1	0.723	49.6	0.687	47.6
32	0.725	53.4	0.722	52.9	0.684	50.7
34	0.726	56.7	0.720	56.2	0.681	53.7
36	0.727	60.0	0.721	59.4	0.680	56.8
38	0.726	63.3	0.720	62.7	0.677	59.8
40	0.725	66.6	0.719	65.9	0.675	62.9
42	0.726	69.9	0.719	69.2	0.673	65.9
44	0.725	73.2	0.720	72.5	0.670	68.9
46	0.724	76.5	0.718	75.7	0.669	71.9
48	0.723	79.8	0.715	78.9	0.668	74.9
50	0.709	83.0	0.497	81.2	0.663	77.9
52	0.461	85.1	0.246	82.3	0.580	80.5
54	0.147	85.8	0.145	83.0	0.338	82.0
56	0.046	86.0	0.099	83.4	0.211	83.0
58	0.035	86.2	0.061	83.7	0.118	83.5
60	0.021	86.2	0.037	83.9	0.081	83.9
62	0.017	86.3	0.027	84.0	0.057	84.1

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.733**
- **in the presence of Triton X-100: 0.736**
- **in the presence of Dowfax 8390: 0.741**

Table A-21: Total *Pseudomonas* Cells Recovered from Column (Series 2)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.793	3.6	0.750	3.4	0.734	3.4
4	0.748	7.0	0.728	6.8	0.713	6.7
6	0.744	10.3	0.721	10.1	0.708	9.9
8	0.742	13.7	0.722	13.4	0.702	13.2
10	0.740	17.0	0.722	16.7	0.694	16.3
12	0.737	20.4	0.721	20.0	0.684	19.5
14	0.735	23.7	0.719	23.3	0.681	22.6
16	0.733	27.0	0.718	26.6	0.680	25.8
18	0.733	30.3	0.717	29.9	0.679	28.9
20	0.732	33.6	0.715	33.2	0.678	32.0
22	0.731	36.9	0.715	36.5	0.676	35.1
24	0.730	40.2	0.716	39.8	0.675	38.2
26	0.728	43.5	0.714	43.1	0.674	41.3
28	0.730	46.8	0.714	46.3	0.674	44.4
30	0.729	50.1	0.712	49.6	0.672	47.5
32	0.730	53.4	0.712	52.9	0.670	50.6
34	0.731	56.7	0.710	56.1	0.668	53.7
36	0.731	60.0	0.711	59.4	0.666	56.8
38	0.730	63.4	0.710	62.7	0.664	59.8
40	0.730	66.7	0.710	65.9	0.661	62.9
42	0.731	70.0	0.711	69.2	0.658	65.9
44	0.732	73.3	0.710	72.4	0.654	68.9
46	0.729	76.6	0.709	75.7	0.652	71.9
48	0.727	79.9	0.707	78.9	0.650	74.9
50	0.714	83.1	0.491	81.2	0.646	77.9
52	0.468	85.2	0.242	82.3	0.563	80.5
54	0.146	85.9	0.141	83.0	0.323	81.9
56	0.049	86.1	0.096	83.4	0.195	82.8
58	0.038	86.3	0.060	83.7	0.097	83.3
60	0.023	86.4	0.038	83.8	0.077	83.6
62	0.018	86.4	0.029	84.0	0.049	83.9

Initial Optical Densities of Microbial Suspensions:

• **in the absence of surfactant:**	**0.737**
• **in the presence of Triton X-100:**	**0.726**
• **in the presence of Dowfax 8390:**	**0.724**

Table A-22: Total *Pseudomonas* Cells Recovered from Column (Series 3)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.812	3.5	0.781	3.4	0.768	3.4
4	0.777	6.9	0.758	6.8	0.748	6.7
6	0.771	10.3	0.751	10.1	0.743	9.9
8	0.770	13.6	0.752	13.4	0.737	13.2
10	0.768	17.0	0.750	16.7	0.728	16.4
12	0.766	20.3	0.751	20.1	0.717	19.5
14	0.762	23.6	0.747	23.4	0.713	22.7
16	0.760	27.0	0.745	26.6	0.711	25.8
18	0.761	30.3	0.745	29.9	0.710	28.9
20	0.760	33.6	0.744	33.2	0.708	32.0
22	0.759	36.9	0.746	36.5	0.707	35.1
24	0.758	40.2	0.744	39.8	0.706	38.2
26	0.759	43.5	0.742	43.1	0.704	41.3
28	0.757	46.8	0.743	46.4	0.706	44.4
30	0.758	50.1	0.741	49.6	0.703	47.5
32	0.758	53.4	0.742	52.9	0.701	50.6
34	0.757	56.7	0.740	56.2	0.699	53.7
36	0.759	60.0	0.741	59.4	0.697	56.8
38	0.757	63.3	0.741	62.7	0.696	59.8
40	0.759	66.6	0.739	66.0	0.694	62.9
42	0.758	69.9	0.739	69.2	0.691	65.9
44	0.758	73.2	0.741	72.5	0.686	68.9
46	0.756	76.5	0.739	75.8	0.683	71.9
48	0.753	79.8	0.735	79.0	0.680	74.9
50	0.738	83.0	0.512	81.3	0.675	77.9
52	0.490	85.1	0.253	82.4	0.591	80.5
54	0.165	85.9	0.149	83.1	0.343	82.0
56	0.052	86.1	0.098	83.5	0.211	82.9
58	0.043	86.3	0.064	83.8	0.108	83.4
60	0.028	86.4	0.041	83.9	0.082	83.8
62	0.020	86.5	0.032	84.1	0.054	84.0

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.765**
- **in the presence of Triton X-100: 0.755**
- **in the presence of Dowfax 8390: 0.758**

Table A-23: Total *Pseudomonas* Cells Recovered from Column (Reduced Loading)

Fraction Collected (mL)	O.D. Blank Solution	Accumulated % of Cells Recovered (Blank)	O.D. Triton X-100 Solution	Accumulated % of Cells Recovered (Triton X-100)	O.D. Dowfax 8390 Solution	Accumulated % of Cells Recovered (Dowfax 8390)
2	0.688	5.4	0.668	5.2	0.642	5.1
4	0.652	10.5	0.649	10.2	0.630	10.1
6	0.646	15.5	0.645	15.2	0.620	15.0
8	0.644	20.5	0.643	20.1	0.613	19.8
10	0.643	25.5	0.642	25.1	0.608	24.6
12	0.641	30.5	0.643	30.1	0.599	29.4
14	0.639	35.5	0.643	35.0	0.595	34.1
16	0.638	40.5	0.641	40.0	0.594	38.8
18	0.636	45.5	0.640	44.9	0.592	43.5
20	0.637	50.4	0.640	49.9	0.593	48.1
22	0.635	55.4	0.638	54.8	0.591	52.8
24	0.634	60.3	0.637	59.7	0.590	57.5
26	0.635	65.3	0.636	64.6	0.589	62.2
28	0.635	70.2	0.633	69.5	0.587	66.8
30	0.633	75.2	0.629	74.4	0.584	71.4
32	0.632	80.1	0.535	78.5	0.581	76.0
34	0.407	83.3	0.339	81.2	0.464	79.7
36	0.248	85.2	0.153	82.3	0.240	81.6
38	0.103	86.0	0.097	83.1	0.151	82.8
40	0.054	86.4	0.065	83.6	0.085	83.4
42	0.024	86.6	0.039	83.9	0.056	83.9

Initial Optical Densities of Microbial Suspensions:

- **in the absence of surfactant: 0.641**
- **in the presence of Triton X-100: 0.647**
- **in the presence of Dowfax 8390: 0.632**

APPENDIX B - Sample Calculations

B.1: Determination of Surface to Volume Ratio in Test Tubes

Surface area available for adhesion $= 2\pi r_{tube} * h$
$= 2 * \pi * (0.8 \text{ cm})(9.4 \text{ cm})$
$= 47.2 \text{ cm}^2$

Volume of liquid $= 5.0 \text{ cm}^3$

S/V $= 47.2/5.0$
$= 9.4 \text{ cm}^2/\text{ cm}^3$

B.2: Determination of Percentage of Cells Adhering to Surfaces

$$\% \text{cells adhered to surface} = [1 - \text{cells in solution}] * 100\%$$

$$= \left[1 - \left(\frac{\text{O.D.}_{\text{cells}} - \text{O.D.}_{\text{no cells}}}{\text{O.D.}_{\text{initial}}}\right)\right] * 100\%$$

Example:

- Initial adhesion of *Rhodococcus* cells (series 1)
- EDM tar
- Triton X-100

$$\% \text{cells adhered to surface} = \left[1 - \left(\frac{0.580 - 0.089}{0.592}\right)\right] * 100\%$$
$$= 17\%$$

B.3: Determination of the Statistical Difference Between Adhesion of Cells to Tars and to Glass

The following method compares the mean values for two sets of data. This method ignores the dependence between the sets of data.

For each set of data, the mean (μ), the variance (σ), and the number of data points (n) are required. Let one set of data be denoted by the subscript 1 and the other set of data be denoted by the subscript 2.

First, a general expression for a pooled estimate of the standard deviation is found:

$$\sigma_p = \sqrt{\frac{(n_1 - 1)\sigma_1^2 + (n_2 - 1)\sigma_2^2}{(n_1 - 1) + (n_2 - 1)}}$$

with $(n_1 - 1) + (n_2 - 1)$ degrees of freedom (Bacon 1992).

The estimated standard deviation between the two means may be determined (Bacon 1992):

$$\left(\text{Estimated Standard Deviation of } (\mu_1 - \mu_2)\right) = \sigma_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

A 95% confidence interval for the difference between the true means may be determined:

$$(\mu_1 - \mu_2) \pm t_{df,0.975} * (\text{Estimated Standard Deviation of } (\mu_1 - \mu_2))$$

where $t_{df,0.975}$ represents the tail area probability points corresponding to the t distribution with df degrees of freedom (Bacon 1992).

Example:

- Initial Adhesion of *Rhodococcus* Cells
- Comparison between EDM tar and blank

$\mu_{EDM} = 40.5\%$; $\sigma_{EDM} = 2.40\%$; $n_{EDM} = 6$

$\mu_{blank} = 48.8\%$; $\sigma_{blank} = 2.71\%$; $n_{blank} = 6$

Thus, $\sigma_p = 2.5\%$

(Estimated Standard Deviation of $(\mu_{EDM} - \mu_{blank})$) = 1.5%

$t_{df,0.975} = 2.228$

The 95% confidence interval for the difference between the means is $(\mu_{EDM} - \mu_{blank}) \pm 3.3$.

B.4: Determination of Void Space Within Packed Column

(a) by eluting water through matrix:

17.6 ± 0.3 mL

(b) by calculation:

Volume of packed column $= \pi * r_{column}^2 * h_{column}$
$= \pi * (0.6\ \text{cm})^2 * 40.0\ \text{cm}$
$= 45.2\ \text{cm}^3$

Volume of packing $= \dfrac{\text{mass of glass beads}}{\text{density of glass beads}}$
$= \dfrac{74.0\ \text{g}}{2.65\ \text{g/cm}^3}$
$= 27.9\ \text{cm}^3$

Void space $= 45.2 - 27.9$
$= 17.3\ \text{mL}$

B.5: Determination of Accumulated Cells Recovered from Column

$$A_n = \sum_{1}^{n} \left(\frac{\text{O. D.}_n * \text{fraction volume}}{\text{O. D.}_{initial} * \text{microbial loading volume}} \right) * 100\%$$

where: n = fraction number
A = total cells accumulated

Example:

- Total *Rhodococcus* cells recovered from column (series 1)
- Triton X-100

$$A_1 = \left(\frac{0.333 * 2}{0.696 * 60} \right) * 100\%$$
$$= 1.6\ \%$$

$$A_2 = A_1 + \left(\frac{0.214 * 2}{0.696 * 60} \right) * 100\%$$
$$= 1.6 + 1.0$$
$$= 2.6\ \%$$

B.6: Determination of the Effective Velocity of Cells in Roller Test Tubes and in Packed Columns

(a) in roller test tubes:

Rotational velocity of roller test tube rack = 12 rpm

$V_{eff(tube)}$ $= (2\pi r)_{tube} * 12$ rpm
$= 2 * \pi * 0.8$ cm $* 12$ rpm
$= 60$ cm/min

(b) in packed column:

$V_{superficial}$ = elution rate of cells/cross-sectional area of column
$= \dfrac{12\ cm^3/min}{\pi(0.6)^2 cm^2}$
$= 11$ cm/min

$V_{eff(column)}$ $= V_{superficial} * V_{column}/V_{void}$
$= 11$ cm/min $* 45.2\ cm^3/17.3\ cm^3$
$= 28$ cm/min

APPENDIX C - Statistical Analyses

Table C-1: Statistical Difference Between Affinity of *Rhodococcus* Cells for Organic Tars and for Glass in the Absence of Surfactants (Series 1)

Tar		Test tube 1	Test tube 2	Test tube 3	Test tube 4	Test tube 5	Test tube 6	Difference Between Tar and Glass
EDM	Final O.D.	0.333	0.351	0.363	0.370	0.368	0.365	3.3
	% cells adhered	44.9	41.8	39.8	38.6	38.9	39.4	
PAA	Final O.D.	0.321	0.342	0.334	0.351	0.341	0.366	3.4
	% cells adhered	47.3	43.8	45.1	42.2	43.9	39.7	
DEV	Final O.D.	0.341	0.353	0.366	0.359	0.371	0.356	3.0
	% cells adhered	43.9	41.8	39.6	40.8	38.8	41.3	
MTL	Final O.D.	0.349	0.378	0.365	0.369	0.356	0.339	3.3
	% cells adhered	40.5	35.5	37.8	37.1	39.3	42.2	
NONE	Final O.D.	0.294	0.318	0.296	0.286	0.287	0.323	
	% cells adhered	49.9	45.8	49.6	51.3	51.1	45.0	

Table C-2: Statistical Difference Between Affinity of *Rhodococcus* Cells for Organic Tars and for Glass in the Absence of Surfactants (Series 2)

Tar		Test tube 1	Test tube 2	Test tube 3	Test tube 4	Test tube 5	Test tube 6	Difference Between Tar and Glass
EDM	Final O.D.	0.370	0.355	0.382	0.367	0.358	0.371	2.3
	% cells adhered	39.3	41.9	37.3	39.8	41.4	39.2	
PAA	Final O.D.	0.383	0.367	0.391	0.372	0.377	0.381	2.2
	% cells adhered	36.6	39.3	35.2	38.5	37.6	36.9	
DEV	Final O.D.	0.353	0.342	0.374	0.361	0.363	0.356	2.4
	% cells adhered	41.2	43.1	37.6	39.9	39.5	40.7	
MTL	Final O.D.	0.378	0.372	0.379	0.381	0.375	0.375	1.8
	% cells adhered	35.6	36.6	35.4	35.1	36.1	36.1	
NONE	Final O.D.	0.284	0.268	0.292	0.291	0.288	0.301	
	% cells adhered	51.6	54.3	50.3	50.4	50.9	48.7	

Table C-3: Statistical Difference Between Affinity of *Pseudomonas* Cells for Organic Tars and for Glass in the Absence of Surfactants (Series 1)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.284	0.266	0.275	5.8
	% cells adhered	51.1	54.2	52.7	
PAA	Final O.D.	0.287	0.294	0.290	4.5
	% cells adhered	50.5	49.3	50.0	
DEV	Final O.D.	0.346	0.337	0.340	4.7
	% cells adhered	41.7	43.3	42.8	
MTL	Final O.D.	0.371	0.362	0.355	5.5
	% cells adhered	35.6	37.2	38.4	
NONE	Final O.D.	0.361	0.353	0.342	
	% cells adhered	37.3	38.7	40.6	

Table C-4: Statistical Difference Between Affinity of *Pseudomonas* Cells for Organic Tars and for Glass in the Absence of Surfactants (Series 2)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.278	0.269	0.284	4.9
	% cells adhered	52.1	53.7	51.0	
PAA	Final O.D.	0.305	0.297	0.286	5.5
	% cells adhered	47.3	48.7	50.6	
DEV	Final O.D.	0.356	0.360	0.371	5.0
	% cells adhered	39.3	38.6	36.7	
MTL	Final O.D.	0.354	0.369	0.371	5.5
	% cells adhered	38.3	35.7	35.4	
NONE	Final O.D.	0.392	0.381	0.376	
	% cells adhered	31.7	33.6	34.5	

Table C-5: Statistical Difference Between Removal of *Rhodococcus* Cells from Organic Tars and from Glass in the Absence of Surfactants (Series 1)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.414	0.429	0.410	7.3
	% cells adhered	32.1	29.6	32.7	
PAA	Final O.D.	0.301	0.292	0.295	6.3
	% cells adhered	50.5	52.0	51.5	
DEV	Final O.D.	0.286	0.304	0.323	9.7
	% cells adhered	53.5	50.5	47.4	
MTL	Final O.D.	0.401	0.398	0.403	6.0
	% cells adhered	34.0	34.5	33.7	
NONE	Final O.D.	0.294	0.303	0.275	
	% cells adhered	51.6	50.2	54.8	

Table C-6: Statistical Difference Between Removal of *Rhodococcus* Cells from Organic Tars and from Glass in the Absence of Surfactants (Series 2)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.420	0.425	0.419	2.9
	% cells adhered	30.4	29.6	30.6	
PAA	Final O.D.	0.304	0.301	0.296	3.1
	% cells adhered	49.6	50.1	50.9	
DEV	Final O.D.	0.302	0.306	0.295	3.5
	% cells adhered	50.4	49.8	51.6	
MTL	Final O.D.	0.396	0.402	0.395	3.0
	% cells adhered	34.2	33.2	34.4	
NONE	Final O.D.	0.298	0.290	0.286	
	% cells adhered	50.5	51.8	52.5	

Table C-7: Statistical Difference Between Removal of *Pseudomonas* Cells from Organic Tars and from Glass in the Absence of Surfactants (Series 1)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.285	0.296	0.282	4.4
	% cells adhered	52.3	50.5	52.9	
PAA	Final O.D.	0.303	0.300	0.294	3.7
	% cells adhered	49.3	49.8	50.8	
DEV	Final O.D.	0.347	0.329	0.342	5.0
	% cells adhered	42.0	45.0	42.8	
MTL	Final O.D.	0.360	0.384	0.379	6.2
	% cells adhered	39.7	35.6	36.5	
NONE	Final O.D.	0.402	0.388	0.391	
	% cells adhered	32.6	34.9	34.4	

Table C-8: Statistical Difference Between Removal of *Pseudomonas* Cells from Organic Tars and from Glass in the Absence of Surfactants (Series 2)

Tar		Test tube 1	Test tube 2	Test tube 3	Difference Between Tar and Glass
EDM	Final O.D.	0.291	0.296	0.301	3.9
	% cells adhered	51.8	51.0	50.1	
PAA	Final O.D.	0.311	0.306	0.315	3.8
	% cells adhered	48.6	49.5	48.0	
DEV	Final O.D.	0.341	0.338	0.344	3.5
	% cells adhered	43.6	44.1	43.1	
MTL	Final O.D.	0.391	0.384	0.378	4.3
	% cells adhered	35.1	36.3	37.3	
NONE	Final O.D.	0.407	0.396	0.411	
	% cells adhered	32.5	34.3	31.8	

Table C-9: Statistical Difference Between *Pseudomonas* Cells Recovered from Column in the Presence of Surfactant and in the Absence of Surfactant

Solution	Mean Recovery	Standard Deviation	Difference Between Blank and Surfactant
Blank	86.4	0.10	
Triton X-100	84.0	0.06	0.2
Dowfax 8390	84.0	0.10	0.2